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DATA ON SEX DETERMINATION IN CATTLE.[1]

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INTRODUCTION.

In a paper from this Station published over twenty-two years ago Russell[2] presented certain statistics which he collected regarding the relation between the sex of a calf and the time in the period of heat (œstrus) at which the copulation occurred which gave rise to it. Data were collected in regard to (*a*) whether service occurred *early* in the "heat," or in the *middle* of the period of œstrus, or *late* in the "heat"; and (*b*) the sex of the resulting offspring. In the published results only the data for "early" and "late" coitus were presented. The figures given were as follows (*loc. cit.*, p. 209):

"82 cows served during the first part of heat produced 31 bull calves and 51 heifer calves.

"76 cows served during the last part of heat produced 42 bull calves and 34 heifer calves."

These figures were admittedly meager. After the paper referred to was published Dr. Russell continued the collection of statistics on this subject but published nothing further. Upon resigning from the Station staff he very kindly turned over to the senior author of the present paper all his original schedules. The complete collection of statistics has recently been subjected to analysis with the results set forth in this paper.

The original schedules contain spaces for the recording of the following information: (1) Breed and age of bull. (2) Breed of

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 42.

[2] Russell, F. L., "Breeding Statistics," Maine Agr. Expt. Stat., Ann. Rept. for 1891, pp. 208, 209.

cow. (3) Age of cow. (4) Date of service. (5) Date of birth of calf. (6) Sex of calf. (7) Weight of calf. (8) "If you know positively, state whether the cow was served in the first, middle, or last part of heat." Space was provided on each blank for recording a year's breeding operations. These schedules were widely distributed among the leading stock breeders of the state, and the inquiry extended over a period of about four years. As in all such studies a portion of the blanks returned were defective and not usable, either through failure to fill in essential points or from mistakes. One of the commonest defects arose from the fact that the bull was allowed to run in the pasture with the cows, thus precluding any possibility of accurate knowledge of the period of œstrus at which coitus occurred. On the other hand the great majority of the returns were filled up in a very careful and painstaking manner, and furnish valuable data on a number of problems in the physiology of breeding which are much more in the foreground of biological interest at the present time than they were when the inquiry was instituted.

All of the breeds of cattle at all common in the eastern United States are represented in the returns. The majority are either grade or cross-bred animals, but a fair proportion represent "pure bred," registered stock, particularly in the case of the dairy breeds.

In the present paper only a single problem will be discussed. In later communications it is hoped to take up certain other problems in the physiology of breeding for which data are available in these returns. The problem here considered may be stated as follows: Is there any relation and if so of what sort, between the time in period of œstrus at which coitus occurs and the sex of the resulting offspring? This is obviously a matter of much practical as well as theoretical importance. There is perhaps no one thing that would be of more vital importance to the stock-breeder than to be able to control, in some degree even, the sex of the animals which he breeds.

The visible manifestations and the duration of œstrus in the cow are rather variable matters. In the older literature, and in the minds of most practical breeders at the present time, no precise distinction is made between the two divisions of the

œstrous cycle designated by Marshall[1] (p. 36) as the *Proœstrum* and *Œstrus* respectively. The combined period is called "heat" in some cases, whereas in others only a portion of the period of marked sexual activity is so designated. This fact, together with the natural variability of the phenomenon itself amongst different individuals, accounts for the rather widely varying statements found in the literature regarding the duration of "heat" in the cow. Leuckart[2] puts the duration for cow and mare at "about" 4 days. Thury[3] implies a rather shorter duration than this. Flemming[4] says regarding the matter: "The frequency and duration of the period of 'rutting' or 'heat' depends upon the age, species, and other circumstances; but it may be said to persist in the domestic animals from 1 to 15 days at the most. The shortest period is witnessed in the cow and sheep and the longest in the bitch. It is sometimes only present from 12 to 24 hours in some non-fecundated animals." Düsing[5] puts the time of active "heat" in cattle at 24–30 hours at the outside. This is the experience of practical breeders generally.

Under these circumstances it is not to be expected that the returns discussed in this paper are absolutely accurate as to the division of the œstrous period into early, middle, and later portions. No sharp delimiting boundaries between these divisions were, or could be, set in the schedules. The matter had to be left to the judgment of the breeder. We have not been able to discover any reason, however, for supposing the error introduced from the indefiniteness of the phenomenon itself to be biased in one direction or the other. There is unquestionably overlapping of the divisions of the œstrous period in the returns, but there is no reason to suppose that this is not as great proportionately in one direction as in the other. The relation

[1] Marshall, F. H. A., "The Physiology of Reproduction," London, 1910.

[2] Leuckart, R., "Zeugung." In Wagner's "Handwörterbuch der Physiologie," Bd. 4, pp. 707–1101, 1853.

[3] Thury, M., "Ueber das Gesetz der Erzeugung der Geschlechter bei den Pflanzen, den Thieren und dem Menschen," Leipzig, 1864.

[4] Flemming, Geo., "A Text-book of Veterinary Obstetrics," New York, 1879.

[5] Düsing, C., "Die Regulierung des Geschlechtsverhältnisses bei der Vermehrung der Menschen, Tiere und Pflanzen," *Jenaische Zeitschr.*, Bd. 17, pp. 593–940, 1884.

between the returns and the true physiological division is probably substantially as follows:

Time of Coitus as Recorded.	*Time of Coitus in Relation to Actual Stage in Œstrus of Individual.*
"Early."	Early + some in middle division.
"Middle."	Middle + some in early stage + an equal number in late stage.
"Late."	Late + some in middle division.

Finally there seems no reason to think that a psychological bias, conscious or unconscious, on the part of the breeders has influenced the returns. The opinions of breeders in regard to sex determination, so far as we have been able to get at them, are as follows: The majority of cattle breeders attach no significance to the time of service as a factor in influencing sex. Either they have never heard of any theory regarding the matter, or if they have are not disposed to attach any significance to it, or to modify or regulate their breeding practice in any way to conform to such a theory. A much smaller number of breeders have a decided opinion on the matter, which they usually believe to be supported in greater or less degree by their own experience. This latter class falls into two groups, about equal in numbers, and about equally dogmatic in their adherence to their particular views. One group believes that in order to secure a preponderance of females service must occur *early* in the "heat," whereas the other group maintains that to reach this end service must be *late* in the "heat." Thus one group offsets the other. Even from the men in these classes, however, the returns bear every evidence of being carefully and honestly made. An occasional somewhat rueful comment may be pencilled on the blank when the writer's theory failed to "work," but further than this preconceptions appear to have played no part.

The material would appear, from the biometrical standpoint, to be particularly favorable on the following accounts:

1. The sampling is strictly random. The different breeds are represented in substantially the proportions in which they

occurred in the State at the time of the inquiry. The statistics do not represent one or even a few large herds, but a large number of small herds scattered all over the State.

2. The original records were made by persons who knew nothing whatever of what use was to be made of them, or anything about what they "ought" to show in order to please or satisfy the person who collected them.

3. The numbers are sufficiently large to reduce the probable error of the sex ratios to reasonably small magnitudes. Of course, still more figures would be desirable, but the data as they stand are by far the most extensive yet collected on the point at issue. In the present state of knowledge regarding sex-determination it is desirable to submit to careful analysis any collection of statistics sufficiently extensive to be worthy the name.

Of course, any one considering the sex-determination problem from the statistical standpoint should be clear as to the nature of statistical evidence in general. Nothing can ever be finally "proved" by statistical evidence alone. All that any amount of statistical data can do is to state a *particular* set of facts, which are a sample, strictly localized in time and space, and restricted in magnitude, out of the whole universe of facts of like kind. If the counting has been correctly done the results from the sample are absolutely and unshakably *true, so far as concerns the sample itself*. Whether or not the sample fairly represents the relations in the whole universe to which it belongs, can never be *absolutely* determined, no matter how large the sample may be. All that can be ascertained is a more or less close approximation to the mathematical probability that the sample gives a just representation of the population or universe from which it came.

The most extensive statistics which have been published on the gross (*i. e.*, unanalyzed) sex ratio in cattle are those of Wilckens.[1] They embrace 4,900 calves and the sex distribution is 2,536 ♂ : 2,364 ♀, giving a sex ratio of 107.3 ♂ : 100 ♀.

[1] Wilckens, M., "Untersuchung über das Geschlechtsverhältniss und die Ursachen der Geschlechtsbildung bei Hausthieren," *Landwirtsch. Jahrb.*, Bd. 15, pp. 611–654, 1886.

Data.

The sex distribution of 480 calves, in relation to the time of coitus in the œstrous period, is exhibited in Table I. Besides the absolute figure the sex-ratios calculated as number of ♂♂ per 100 ♀♀ and the percentages of ♂♂ and ♀♀ and their probable errors are given.

TABLE I.

SHOWING THE NUMBER OF CALVES OF EACH SEX, AND THE SEX RATIOS, FOLLOWING COITUS AT SPECIFIED TIMES IN THE ŒSTROUS PERIOD.

Time of Coitus.	Total Off-spring.	Sex of Offspring.		Percentage of		♂♂ to 100 ♀♀
		♂♂	♀♀	♂ Births.	♀ Births.	
Early in heat	248	123	125	49.60 ± 2.14	50.40	98.4
Middle of heat	125	67	58	53.60 ± 3.01	46.40	115.5
Late in heat	107	65	42	60.75 ± 3.19	39.25	154.8
Totals, all periods	480	255	225	53.13 ± 1.54	46.87	113.3

From this table the following points are to be noted:

1. The proportion of male births increases steadily with the later occurrence of coitus in the œstrous period.

2. Comparing the extremes there are more than 10 per cent. more males produced when coitus is late in heat than when it is early in the œstrous period.

3. Taking the three groups together the total figures give a sex-ratio intermediate between those exhibited in the extreme groups.

Now to consider the important question: Are the differences between the different groups shown in Table I., statistically significant, or are they such as probably arise from the inevitable "error" of random sampling? To get light on this point recourse must be had to the probable errors of the sex-proportions.

The probable errors given in the percentage column for the male births are calculated from the usual formula[1] for the probable error of a proportion or percentage, viz:

$$\text{P.E.} = .67449\sqrt{\frac{p \cdot q}{n}},$$

[1] See G. Udny Yule's "An Introduction to the Theory of Statistics," London, 1911 (Griffin & Company), for a very clear discussion of this and other probable error formulæ.

where p denotes the proportion of "successes," q the proportion of "failures," and n the number in the sample.

Remembering that between two uncorrelated events the probable error of a difference is the square root of the sum of the squares of the probable errors of the quantities between which the difference is taken, we have the results set forth in Table II. From this table it is possible to form a first judgment as to the statistical significance of the raw data of Table I.

TABLE II.

SHOWING THE PROBABLE ERRORS OF THE DIFFERENCES BETWEEN CERTAIN OF THE SEX-RATIOS OF TABLE I.

Groups Compared.	Difference.	Probable Error of Difference.	Difference. / P.E. Diff.
Per cent. ♂ births "late in heat"—"early in heat"	11.15	±3.84	2.90
Per cent. ♂ births "late in heat"—"middle of heat"	7.15	±4.39	1.63
Per cent. ♂ births "middle of heat"—"early in heat"	4.00	±3.69	1.08

From this table we note that in the case of the extreme groups the difference is 2.9 times its probable error. This means[1] that if the time of coitus in relation to œstrus were absolutely without influence on sex an excess of 11 per cent. of males such as is observed in the extreme case would only happen 5 times in every hundred that the matter was tested with statistical samples of the present size. Or, put in another way, the odds are 19 to 1 against the excess of ♂ births in "late in heat" matings being due to "chance" (*i. e.*, to errors of sampling) on the basis of probable errors here used. These are distinctly "long odds."

Can the probable errors calculated by the formula used above be relied upon to give correct results on data like the present? They assume normality of the error distributions. This assumption is practically justified in the present instance. The material may, however, be dealt with according to the method given by Pearson.[2] The present case may be regarded as falling

[1] This statement regarding probabilities assumes that the errors of sampling for this material follow substantially the normal or Gaussian curve of error See below.

[2] Pearson, K., "On the Influence of Past Experience on Future Expectation," *Phil. Mag.*, March, 1907, pp. 365–378.

in the category of estimating future expectation on the basis of experience furnished by a previous sampling. Pearson (*loc. cit.*) shows that only when a first sample is indefinitely larger than a second can we with entire accuracy take the probable error of the latter as $.67449\sqrt{mpq}$, when p and q are the chances of success and failure respectively in the former. He further shows that when a first sample n gives a percentage value of the character equal to $\bar{p}$, a second sample of m individuals may be expected to give

$$100\,\bar{p} \pm 67.449\sqrt{\bar{p}\bar{q}\left(\frac{1}{m}+\frac{1}{n}\right)} \text{ per cent.}$$

In the present instance we may take the cases of service early in heat as giving a "first sample" for the sex ratio in cattle, and then proceed to determine whether the groups served in the middle of heat and late in heat are to be regarded as random samples from the "early in heat" population.

We then have for the cases of service in middle of heat

$$\begin{aligned} \bar{p} &= .496, \\ \bar{q} &= .504, \\ m &= 125, \\ n &= 248, \end{aligned}$$

whence the expectation for the percentage of ♂ births from cows served in the middle of heat, provided time or service had *no* influence on sex, any deviation being due to errors of sampling, is 49.6 ± 3.7. Or, as a result purely of random sampling a second sample from the "early in heat" population would be expected to show as often as not a ♂ sex percentage anywhere between the limits 45.9 and 53.3.

Actually the observed percentage of males for service in "middle of heat" was 53.6, practically the same as the upper limit given.

For cases of service in last of heat we have $m = 107$, and the other values as before. Whence it is deduced, by the same reasoning as before, that the expectation for the percentage of male births from cows served *late* in the period of heat, provided time of service had *no* influence on sex, any deviation being due to errors of random sampling, would be 49.6 ± 3.9.

This means that a random sample of the size of this "late in heat" groups, from a population having the sex ratio exhibited by the "early in heat" sample, would be expected as often as not to give a male sex percentage within the limits 45.7 and 53.5.

The observed percentage of male births in the "late in heat" lot was 60.75. This value is well outside the limits, and the probability against its being a result of random sampling is considerable. It is not, however, so great as to amount to certainty. The standard deviation of the error distribution for random samples of the size of this "late in heat" lot is approximately 6.2. Taking the rough rule that for practical purposes the half range as either side of the mean is 3 σ, it would give an upper limit to the curve of 67.9. The observed 60.75, while getting well out towards the range end, still lacks something of actually reaching that point, to say nothing of going beyond.

In general, the data presented appear to warrant the following conclusions: *So far as the present statistics are concerned there is an increasing proportion of male births as the time of service approaches the termination of the œstrous period.* This increase amounts to approximately 10 per cent. in the extreme case, and to not quite half this in the intermediate case. Statistically considered the difference in the extreme case is *probably* significant (*i. e.*, not due to errors of random sampling). The probability that the observed increase in the proportions of male births is a real and biologically significant phenomenon, while not enormously great, is nevertheless of an order which one would not hesitate to act upon in the practical affairs of life.

So much for the bare facts. We have now to consider the possibilities as to the cause of the observed relation. *Apparently* the increase in male births is associated with time of service, but before accepting such a conclusion it is important to determine whether some other variable factor may not be influencing the results. The first possibility which suggests itself is that there may be a difference in age distribution of the animals in the three lots and that in some manner this has affected the result. In the ideal experiment of this sort of course the parents used should be of the same average age in the different groups.

The age distribution of the animals involved in these statistical

returns has been examined with the results shown in Tables III. and IV. Table III. gives the actual frequency distribution, and Table IV. the constants calculated therefrom. It should be said that in these distributions the parents are weighted with their fertility. That is, each individual appears once for each offspring. This seems the fairest manner in which to deal with the problem, for comparisons such as are here indicated.

TABLE III.

SHOWING THE AGE DISTRIBUTION OF THE ANIMALS IN THE SEVERAL GROUPS.

Age in Years.	Service Early in Heat.		Service Middle of Heat.		Service Late in Heat.	
	No. of ♂♂.	No. of ♀♀.	No. of ♂♂.	No. of ♀♀.	No. of ♂♂.	No. of ♀♀.
1– 1.9	71	7	44	4	25	2
2– 2.9	68	26	26	15	49	12
3– 3.9	39	31	16	11	13	15
4– 4.9	8	23	4	13	2	13
5– 5.9	17	16	5	12	1	8
6– 6.9	2	30	1	10	2	12
7– 7.9	—	27	—	7	—	12
8– 8.9	—	13	—	10	—	11
9– 9.9	—	8	—	6	—	8
10–10.9	—	16	—	6	—	5
11–11.9	—	7	—	2	—	2
12–12.9	—	4	—	6	—	1
13–13.9	—	2	—	—	—	1
14–14.9	—	0	—	—	—	—
15–15.9	—	1	—	—	—	—
Totals[1]	205	211	96	102	92	102

From these distributions the constants of Table IV. have been calculated by the ordinary biometric methods.

TABLE IV.

SHOWING THE VARIATION CONSTANTS FOR THE AGE OF BREEDING CATTLE.

Group and Sex.	Mean, Years.	Standard Deviation, Years.	Coefficient of Variation, Per Cent.
Service early in heat—♂♂	2.71 ± .06	1.21 ± .04	44.7
Service middle of heat—♂♂	2.49 ± .08	1.16 ± .06	46.6
Service late in heat—♂♂	2.53 ± .07	.93 ± .05	36.6
Service early in heat—♀♀	6.16 ± .14	2.95 ± .10	47.9
Service middle of heat—♀♀	6.15 ± .20	3.04 ± .14	49.5
Service late in heat—♀♀	6.15 ± .18	2.73 ± .13	44.4

[1] The differences between these and the offspring totals are due in the main to failures in individual cases to record the age of an animal in the schedules. In small part they are due to multiple gestation.

Obviously there are no differences in age distribution of sufficient magnitude to cause the observed differences in sex-ratios among the three groups. The males used in the three groups are of very nearly the same mean age. The same is true to an even more marked degree of the females of the three groups. It would be difficult to collect a more homogeneous lot of statistics than the present one, so far as concerns age distribution of the individuals in the several samples.

We have not been able to discover any other factor which could account for the observed differences in the sex-ratios described above. By the most careful tests which we have been able to apply the material appears to be statistically homogeneous except in respect to the time of service.

Some of the individual records are of interest. The following schedule from Mr. C. E. Clifford may be cited in particular. He was a breeder of registered Jersey cattle and carefully followed a regular system of service in relation to time of heat. This method in his own words was as follows: "Cover cow twice *immediately* upon discovery of heat and keep her separate from all [other cattle] until heat is entirely passed." The sex distribution for 14 calves born in the year for which the return was made was 2 ♂♂ and 12 ♀♀.

Discussion.

It will have been noted that the results set forth above support, in essential features, the theory of Thury (*loc. cit.*) as to sex determination in cattle. Thury's original paper was published in French under the title "Memoir sur la loi de production des sexes chez les plantes, les animaux et l'homme." It immediately attracted the attention of biologists and was critically reviewed and discussed in the *Zeitschrift für wissenschaftliche Zoologie* (Bd. XIII., Heft 4) by Pagenstecher. He later translated Thury's paper into German, added appendices, etc., and it is this German translation which has chiefly been cited by later workers. The essential features of Thury's theory, so far as concerns mammals normally bearing a single young at birth, were set forth in the following words (Thury, *loc. cit.*, p. 16).

"Das Geschlecht hängt ab vom Grade der Reifung des Eies im Augenblicke, wo es von der Befruchtung getroffen wird.

"Das Ei, welches, wenn es befruchtet wird, noch nicht einen gewissen Grad der Reifung erreicht hat, giebt ein Weibchen; ist dieser Grad der Reifung überschritten, so giebt das Ei, wenn es befruchtet wird, ein Männchen.

"Wenn zur Zeit der Brunst, ein einziges Ei, vom Eierstock abgelöst, langsam durch den Geschlechtsapparat herabsteigt (Thiere, welche ein Junges gebären), so genügt, es dass die Befruchtung am Anfange der Brunst statthabe, um Weibchen zu zeugen, und am Ende, um Männchen zu zeugen, indem die Umwandlung (vire) des Zustandes des Eies normal während der Dauer seines Durchganges durch den Geschlechtskanal stattfindet."

Curiously enough this theory, while exciting much interest at the time of its appearance, has never been given any adequate experimental-statistical test. The only experimental evidence which Thury himself offered in its favor was that obtained by a friend Cornaz[1] which was so brilliantly confirmatory as to arouse suspicion in the minds of all subsequent workers, with the result that it has never been regarded as of any particular worth. At the best it was ridiculously inadequate statistically. Cornaz states that by following Thury's directions as to time of service he obtained 22 female calves in unbroken succession. In 7 cases he desired and obtained male calves, the service in these cases being late in the heat.

Düsing (*loc. cit.*) discussed Thury's theory at great length, and pointed out in the following words one of its obviously great weaknesses (p. 16): "Indessen ist diese eigentliche Thury-sche Theorie in sofern nicht richtig, als immer nur ein gewisser Überschuss des einen oder andern Geschlechtes erwartet werden kann." Thury postulated *complete* determination.

Later writers have dismissed Thury's theory with rather scant consideration. Thus F. H. A. Marshall (*loc. cit.*, p. 630) says: "Thury claimed that he could regulate the sexes in cattle by allowing coitus only at the beginning or at the end of œstrous periods, . . . but other investigators have failed to establish Thury's conclusions." Morgan[2] (p. 393) says: "Thury based

[1] Published as a part of Thury's paper (*loc. cit.*), pp. 17 and 18.

[2] Morgan, T. H., "Experimental Zoölogy," New York, 1907.

based his conclusions on 29 experiments with cows, in which union took place at the beginning or at the end of the period of heat. Others have failed to confirm his conclusions, and contradictory results have been obtained with rabbits, and hens." Neither Marshall nor Morgan give any detailed references to the literature.

F. R. Marshall[1] evidently has Thury's theory in mind in the following statement but has apparently received the tradition in slightly garbled form: "The commonest idea about sex determination is that females bred at the beginning of the period of heat produce male offspring. Other notions are based on the same supposed principle, namely, that an ovum fertilized while immature produces a male; maturity is supposed to be in proportion to the age of the ovum and the nutritive condition of the dam." He then proceeds (on grounds it must be said which are strictly *a priori*) to demolish all such notions.

So far as we have been able to discover the following are the only experimental tests which have ever been accorded Thury's theory as regards cattle breeding, which at all adequately fulfill the conditions necessary for a critical test. Supposed tests of the theory carried out with plants, multiparous mammals, birds, etc., will not be discussed here, because it seems to us either (*a*) obvious *a priori* that the theory could not possibly apply, in the nature of the case, to some of the organisms and forms of reproduction which Thury and subsequent workers attempted to include in its purview, or (*b*) that the so-called tests of the theory were in many instances absolutely uncritical and could not have been expected under the most favorable circumstances to have yielded any really significant results one way or the other. The tests with cattle, which if carefully carried out must yield critical evidence, are, as has been said, meager enough in number and magnitude. The first were made shortly after the publication of the theory at the agricultural academies at Proskau and Eldena.[2] The results were as follows:

[1] Marshall, F. R., "Breeding Farm Animals," Chicago, 1911.

[2] *Annalen der Landwirtschaft*, Jahrg. 23, Bd. 46, p. 271, 1865. For the facts regarding these and the following tests we are indebted to Düsing's (*loc. cit.*) memoir.

Time of Service.	Sex of Young. ♂	♀
Early in heat	8	10
Late in heat	4	1

From these figures it was concluded that the theory in the bald form proposed by Thury was untenable.

The matter was again tested in Waldau and Eldena[1] with the following results:

Time of Service.	Sex of Young. ♂	♀
Early in heat	3	10
Late in heat	1	1

The last experimental test of the matter of which we have been able to find any report (except that of Russell, *loc. cit.*) is that of Touchon[2] who obtained the following results.

Time of Service.	Sex of Young. ♂	♀
Early in heat	0	11

These figures are very far from extensive. In view of the great practical importance of the matter, however, one would have thought that they would have acted as a stimulus to more adequate researches.

It is worth while to put together all the data regarding the relation of time of service to sex in cattle. We have the following figures:

Time of Service.	Sex of Young. ♂	♀	♂♂ : 100 ♀♀
Early in heat	134	178	75.3
Middle of heat	67	58	115.5
Late in heat	77	44	175.0
Totals	278	280	

These figures are suggestive. While they certainly demonstrate that Thury's theory in its original form is untenable, on the other hand, they strongly indicate, though they admittedly do not absolutely *prove*, that the sex ratio in cattle may be modified by controlling the time of service in relation to the œstrous period. We would call attention to the fact that the

[1] *Annalen ner Landwirtschaft*, Wachenblatt, 1866, p. 461.

[2] *Agronomische Zeitung*, 1865, p. 519.

totals give approximately a 1 : 1 ratio, as on *a priori* grounds they should.

Granting the fact that the sex ratio in cattle does change as the time of service changes, what is the biological basis of the phenomenon? Thury's idea was that the sex of the offspring was determined by the relative staleness of the ova. In making this hypothesis it may be assumed either that ovulation occurs early in œstrus entirely, or as a more or less regular process throughout the œstrous period. Unfortunately no exact and thorough studies have been made on the physiology of reproduction in the cow. As has been pointed out by Marshall (*loc. cit.*, p. 316) the fact that artificial insemination has been successfully practiced with cows would indicate that ovulation is independent of coition. In a later paragraph that author states that: "There can be little doubt that in the great majority of mammals ovulation, as a general rule, occurs regularly during œstrus." This would certainly seem to be the case in the cow, since our statistics show that pregnancy may follow coitus at any time during the œstrous period. It has lately been contended by Jentsch,[1] on rather meager evidence, that cows even when served out of the œstrous period will in some cases become pregnant. The duration of life of the unfertilized egg in the uterus is not known for the cow. It has recently been shown by Lewis[2] to be distinctly brief in the case of the sow. He states as the result of numerous observations and experiments that the ovum "does not retain its vitality for more than a few hours after being liberated from the Graafian follicle." But if in the cow ovulation begins at or very near the beginning of œstrus, as seems certain from the returns in some of our schedules, where pregnancy followed a coitus within one or two hours of the beginning of œstrus, then it can fairly be considered as probable that the average age of fertilized ova following coitus late in heat will be somewhat greater than that of the ova fertilized early in heat. Nothing more than an average difference can be claimed (*i. e.*, not every individual ovum fertilized late in heat will be older

[1] Jentsch, A., "Ueber Befruchtung ausserhalb der Brunstzeit beim Rind," *Jahrb. wiss. prakt. Tierzucht.*, Bd. 6, pp. 441–444, 1911.

[2] Lewis, L. L., "The Vitality of Reproductive Cells," Oklahoma Agr. Expt. Sta. Bulletin 96, pp. 1–47, 1911.

than every individual fertilized early) and it is not possible to make any accurate estimate of what the difference amounts to. However, until some more definitely related factor is found it may be tentatively concluded, purely as a working hypothesis, that the observed changes in the sex ratios are correlated with changes in the relative freshness (or staleness) of the ova at the time of fertilization.

The hypothesis that the metabolic condition of the ovum when fertilized may influence the sex of the offspring finds support in the recent work from Richard Hertwig's laboratory. Hertwig[1] in a series of studies on frogs found that over-ripeness of the eggs was associated with a preponderance of males, extending in favorable cases to the production of 100 per cent. males. These results were criticized on several grounds. The experiments, however, have been very carefully repeated and extended by a student of Hertwig's, Kuschakewitsch,[2] who obtained similar results. The experiments were criticized by Morgan first on the ground of differential mortality, and second on the ground of differential fertilization, but Kuschakewitsch was able to show that the results were not open to criticism on these grounds. Whatever the explanation the *facts* brought out by Hertwig and Kuschakewitsch must certainly be accepted. The procedure which they followed led to a great preponderance of males. Miss King[3] has also shown that it is possible to modify significantly the sex-ratio by changing the metabolic condition of the eggs.

In the last few years a considerable body of evidence has accumulated showing that sex determination is primarily a matter of inheritance. It is not necessary to review the voluminous literature here. Essentially two points have been demon-

[1] Hertwig, R., "Ueber das Problem des sexuellen Differenzierung," *Verhandl. deutsch. Zool. Ges.*, 1905; "Weitere Untersuchungen über das Sexualitätsproblem," *ibid.*, 1906; "Weitere Untersuchungen," etc., *ibid.*, 1907.

[2] Kuschakewitsch, S., "Die Entwicklungsgeschichte der Keimdrüsen von *Rana esculenta*. Ein Beitrag zum Sexualitätsproblem," *Festschrift f. Hertwig.*, Bd. 2, 1910.

[3] King, Helen Dean, "Studies on Sex Determination in Amphibians, IV. The Effects of External Factors, Acting Before or During the Time of Fertilization, on the Sex Ratio of *Bufo lentiginosus*," BIOL. BULLETIN, Vol. XX., pp. 205–234, 1911. See also others paper in the same series.

strated. On the one hand the cytologists have shown for many forms that certain chromosomes are definitely associated with sex differentiation. On the other hand, the geneticists have shown that in a considerable number of forms the phenomenon of sex-linked inheritance is exhibited, a phenomenon which has as yet received no more cogent explanation than the obvious one that sex differentiation depends upon germinal factors, and that these behave in inheritance in accordance with Mendelian principles.

No one could rate higher the evidence that sex is determined primarily by inheritance than does the writer. But it is idle to deny that there is also a large and increasing body of critical evidence indicating that, in one way or another, sex ratios may be modified experimentally, and to some degree indeed controlled. Any adequate hypothesis of sex-determination must account both for the facts indicating innate pre-determination in the germ cells before fertilization, and also those indicating the modifying influence of external factors. Because one of these sets of facts is true does not mean that the other set is necessarily false, and should be forthwith annihilated (if possible) by destructive criticism. Such definite facts cannot be mutually exclusive. It would seem that a logical view of the case is that while sex is, in many cases at least, *primarily* determined by innate hereditary causes, nevertheless external factors, acting at the appropriate time, may in some instances modify the effect of the innate factors. This is essentially the same conclusion as is reached by Schleip[1] in his recent extensive and critical review of the literature in this field.

It is not difficult to conceive how such results could be brought about physiologically. The evidence indicates that particular chromosomes are concerned with the hereditary determination of sex. Furthermore a broad survey of the experimental work in the modification of sex-ratios by external conditions indicates that the most effective agencies in bringing about such modifications are those which directly affect the metabolic condition of the germ cells (*e. g.*, staleness, extraction of water, etc.). Why

[1] Schleip, W., "Geschlechtsbestimmende Ursachen im Tierreich," *Ergeb. u. Fortschr. Zool.*, Bd. III., pp. 165–328, 1912.

may not the sex chromosomes be affected by these agencies along with the rest of the germ cell to a sufficient degree to modify their specific effect? As has recently been pointed out:[1] "There are two ways of looking at the relationship between sex chromosomes and primary and secondary sexual characters on the assumption already made that the differential factor is essentially quantitative in nature. On the one hand it may be assumed, as it has been in most discussions of the subject, that the *X*-chromatin acts in a *positive* way, if at all, in the determination of sex. On this view two 'doses' of *X*-chromatin, in some manner not fully understood, positively determine the development of female characteristics. It has been argued that since the female is primarily anabolic in tendency, as pointed out many years ago by Geddes and Thomson, the determination of the female sex by a 'plus' condition in respect of chromatin may be explained on the assumption that *X*-chromatin is a sort of 'tropho-chromatin' in which reside the energy potentialities of the organism.

"There are two difficulties which confront this interpretation. The first is that the female characters cannot be regarded as an extension or intensification of those of the male. Rather the contrary is true. The male almost universally represents a higher degree of specialization and differentiation in development than the female. Another difficulty is found in the phenomenon of hermaphroditism.

"I would suggest that both of these difficulties may be overcome and all of the facts be better interpreted by assuming that *X*-chromatin is not a positive cause of sex differentiation but rather is an *inhibitor* of the development of male sex characters. It is a well-known fact that in the vertebrates, where the embryology of the genital organs have been most carefully studied, the male condition represents a more extended and advanced degree or stage of development than the female. The system is in every part homologous in male and female, but the latter appears objectively to have been arrested in a development which, without such arrest, would have led to the same result

[1] Pearl, R., in as yet unpublished lectures on the "Biology of Sex," delivered before the Graduate School of Agriculture, Lansing, Michigan, July 1–6, 1912.

as is seen in the male. The same consideration applies to the male and female germ cells. The ovum is much less widely differentiated from the primordial indifferent germ cells than is the spermatozoön. Now if it be assumed that two 'doses' of this specific *X*-chromatin serve to inhibit completely the development of 'maleness,' while one 'dose' is insufficient to do this, but allows the male characters to develop, we shall have, it seems to me, a more satisfactory interpretation than is gained by looking at the matter in the reverse way.

"This viewpoint would explain why it is that castrated males do not, save possibly in exceptional circumstances, take on female characters. On the other hand, according to Goodale's observations, castrated females do take on male characters. Further an explanation is found for the numerous cases in which a female in old age or after disease takes on male characters.

"On this view hermaphroditism becomes analogous to the phenomenon of the retention of larval characters in development it being assumed that there is one 'dose' or less of *X*-chromatin in such cases."

On either of these hypotheses as to the sex-determining action of the *X*-chromosomes it is not difficult to conceive how any change in the general metabolic condition of the germ cells might modify the sex ratio. The *X*-chromatin would presumably be affected along with the rest of the cell and the relative potency of its sex-determining factors changed.

In conclusion we wish our position in regard to the results set forth in this paper to be clearly understood. It is not contended or supposed by the writers that the time of service in relation to the period of heat *absolutely controls* the sex of the subsequent offspring. It is believed, however, that the facts set forth show, with a considerable degree of probability, that the sex ratio in cattle can be to some extent *modified* by controlling the time of service. But the amount of such observed modification is not so great that the matter can be tested with a few individuals. There is every reason to believe that any effect would only appear in fairly comprehensive statistics. The matter is one of much practical consequence to the stock breeder. Because this is so we would caution the reader against misinter-

preting the results of this paper. A trial on a half dozen individuals will not in any sense whatever adequately test the accuracy of the results set forth in this paper. Nor will a breeding experiment with any other animal than the cow. If any person chooses to generalize from the data of this paper that in animals in general, or indeed in any other animal than the cow, time of service and sex of offspring are causally related, the responsibility for such generalization must be *entirely* his, not ours. We have studied statistics for cows in regard to this problem, and as yet no others, and have tried to make clear just what these data show. Beyond this solid ground of fact we do not care to venture, particularly so far as concerns the practical application of these results by the breeder.

Time of service is very evidently no absolute determining factor for sex in cattle. On the other hand the probability that the sex ratio can be changed by careful attention to this matter of time of service is sufficiently great, in our judgment, to warrant any man in modifying his breeding practice in accordance with it, particularly since in so doing he will be incurring no added risk of any kind. In the every-day affairs of life in regard to business, investment of funds, and the like, practical men every day undertake courses of action on the basis of probabilities much smaller than that in favor of getting an increased number of males if cows are served late in the heat. The practical cattle breeder in most cases would like, if he could get it, an excess of female calves. All the evidence at hand warrants the belief that by taking care that cows are served *as soon as possible* after the onset of heat there will be some reduction in the proportion of male calves born. In short, the facts set forth in this paper warrant the breeder in paying attention to the time of service in his cattle breeding operations, but he must not suppose that by so doing he can absolutely control the sex of the offspring, or even approach measurably close to absolute control. He can at best merely modify, over a period of years, the sex ratio in greater or less degree, in the direction which he desires.

SUMMARY.

In this paper statistics collected some years ago at the Maine Agricultural Experiment Station in regard to the relation between

time of service in the œstrous period and the sex of the subsequent offspring in domestic cattle are subjected to biometric analysis. These statistics are much more extensive than any which have hitherto been collected for the study of this problem in cattle. It is shown:

1. That as the time of coitus approaches the end of the œstrous period there is a progressive increase in the proportion of male young born.

2. That in the extreme case this increase in the proportion of male births is probably statistically significant and not to be attributed to errors of random sampling.

3. That these modifications of the sex ratio cannot be attributed to age differences or to any other factor yet suggested.

A possible explanation of the results and their practical bearings are discussed.

VARIATIONS IN THE SIZE OF CHROMOSOMES.

FRANK A. HARTMAN.

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I. INTRODUCTORY.

From the time that the importance of germ cell chromosomes was first realized, the mass of investigation on this subject has dealt with the development of the chromosomes. Practically nothing on a comparison of chromosomes of the same species has been published. Therefore these results are given that they may throw some light on the behavior of chromosomes.

The observations in this paper, although somewhat limited, suggest interesting theoretical considerations.

Further investigations are being made by the author to test these ideas.

II. MATERIAL.

All of the material is of the species *Schistocerca americana*, with the exception of one specimen which is *S. alutacea*. A brief description of the material follows.

Young Nymphs.—Out of a number of nymphs, collected at the same time and place near the Kansas University campus, nine were taken at random. These were probably of the same age, for they were of approximately equal size, ranging from 12 to 14 mm. in length.

Older Nymphs.—Two of them, one 26 mm. long, wing stubs 6 mm.; the other 33 mm. long, wing stubs 9 mm. Both were collected by W. R. B. Robertson in Dickinson Co., Kansas.

Adults.—Two were furnished by Mr. Robertson from the locality mentioned above. Three were furnished by the Kansas University Zoölogy Department, two of them being *S. americana* and the other *S. alutacea*. The average measurement of dried adult specimens was 44 mm. exclusive of wings.

All material was fixed with Flemming and stained with Heidenhain's iron hæmatoxylin.

III. Observations.

1. *Comparison of Chromosomes.*

(*a*) *Chromosomes of Nymphs Compared with Those of Adults.*—The greater size of the chromosomes of adults as compared with those of nymphs is most easily seen in the first spermatocyte stages, as there the chromosomes are the largest of any period in their history. Great contrast in the size of chromosomes appears in both metaphase and anaphase, provided the nymphs are young enough. The nymphs, showing the greatest contrast were less than one third grown.

In first spermatocyte metaphase the chromosomes of young nymphs occur more in the spherical or short rod form than as rings or twisted rods which in adults are common. Large chromosomes are usually doubled or coiled and as there are not so many large ones in the nymph cells naturally the number of these complicated forms is smaller.

A large number of drawings were made as carefully as possible with a camera lucida. A few of these were selected in illustrating this paper. They are all typical of the material studied.

The twelve chromosomes within a cell were numbered according to their size. In almost every case the corresponding chromosomes of adult cells were larger than those of young nymph cells. The exceptions to this, although few, occurred among the chromosomes of smaller size (see Plate I., Figs. 1 and 2, 3 and 4, 5 and 6, 15 and 16). In some instances there was great contrast in size (see Plate II., Figs. 25 to 32).

In first spermatocyte anaphase the same comparison holds

good with the exception of the chromosome form. The V- or U-shaped chromosomes of young nymphs are shorter and thicker than those of adults (see Figs. 33 to 47, Plate II.).

In the second spermatocyte metaphase there is a repetition of the size differences discussed in connection with the first spermatocyte (see Figs. 48 to 55, Plate III.). In connection with this it should be stated that almost invariably the cells of the young nymphs were smaller than those of adults.

(*b*) *Comparison of Chromosomes of Different Cells of the Same Individual.*—One of the most interesting conditions appeared in different cells of the same individual. Chromosomes which correspond as the number ones of the different cells show in many cases variations in size (compare corresponding chromosomes of the groups in: Figs. 1, 3, 6, 8, 9, 11, 14, 16; Figs. 2, 4, 5, 7, 10; Figs. 12, 13; Figs. 17, 19, 22, 24, 25, 27, 30, 32; Figs. 18, 23; Figs. 20, 21; Figs. 26, 28, 29, 31; Figs. 56, 57; Figs. 58, 59; Figs. 60, 61, 62, 63). In some instances this difference in size is very striking (see ones in Figs. 5 and 7; nines in Figs. 12 and 13; ones, twos, and threes in Figs. 22 and 24; and many others which are evident by a comparison of the figures).

A great number of cells were studied to find whether this size variation was exceptional. The variation was not unusual, but very common.

The first spermatocyte anaphase presented like differences in size (see Figs. 33, 35, 37, 39, 41, 43, 47; Figs. 34, 38, 40, 42).

Chromosmes in the second spermatocyte metaphase show similar variations (Figs. 48, 50, 55, 49, 51, 54).

The second spermatocyte anaphase chromosomes are just as variable (Figs. 64, 65, 66, 67).

There is no doubt that corresponding chromosomes of the same individual show a tendency to vary in size. This tendency becomes more marked in certain cells.

2. *Causes of Chromosome Size Variation.*

(*a*) *Unequal Growth.*—During early prophase of the first spermatocyte it is well known that the chromosomes appear as groups of granules, which may be more or less clearly distinguished from each other.

One slide which had most of the stain removed contained a number of cells of the early prophase with only one group of granules showing distinctly. The other granules were either very faint or else did not show at all. The granules of this group varied in size and number and in many cases were in the process of division (Figs. 69, 70, and 75 to 80). When the cells grew older, as could be determined by the increase in size of the cell and the appearance of the spiremes of the other chromosomes, the granules of this group began to come closer together and finally fuse (see Figs. 71 to 74 and 82).

The body produced by this fusion is unquestionably a chromosome because it is made up of granules which have the power of division. It is not the accessory chromosome for the accessory is present in practically every cell containing it.

The chromosome appearing in this series of observations seems to vary in size as commonly found among corresponding chromosomes of the same individuals (described above) (compare Figs. 73 and 74).

. The number of granules in each group where no fusion had commenced was as follows: 7, 9, 9, 14, 9, 10, 8, 9, 11, 10—between 9 and 10 seemed to be the average. The fact that some granules had not divided could account to a certain extent for the differences in number, but not in every case, as Fig. 70, in which but one granule is undivided would give but 10 granules if this were divided. An examination of the other groups will show like discrepancies. Therefore the number of granules varies independently of their condition of division.

The variation in the size of the granules in cases where fusion had not occurred, was considerable. Even where all of the granules had divided they varied in size (see Fig. 75, as well as many of the other groups figured). This is true whether we compare granules of the same cell or of different cells (see Figs. following 68 and 75).

(*b*) *Unequal Division.*—In some of the lateral views of the first spermatocyte a few small chromosomes were found dividing unequally (Figs. 85 to 91). One cell contained two chromosomes dividing unequally (Fig. 88). Small chromosomes were found dividing unequally in the second spermatocyte (Figs. 83 and 84).

There is no question then that unequal division does sometimes occur in chromosomes.

IV. Discussion.

1. *Chromosome Variations and Their Causes.*

The difference in size of chromosomes of nymphs as compared to adults depends upon the age of the nymphs. It also bears a direct relation to the size of the cells, the smaller cells of nymphs containing the smaller chromosomes.

Cells of young nymphs are dividing more rapidly than are those of adults, for that reason they probably have not the chance to grow so large. In addition to this the body cells in young animals undoubtedly develop at the expense of the germ cells because it is more important that the body organs become established before the germ cells are brought into activity. Then when the body has almost reached its growth the germ cells get their share of nutrition and become larger as do the chromosomes.

A study of the growth stages of a corresponding chromosome, in the same individual shown in Figs. 68 to 82, has demonstrated that the number and size of the granules differ. Either is sufficient cause for a variation in size of corresponding chromosomes.

Unequal division likewise produces unequal chromosomes.

These two facts lead us to expect unequal corresponding chromosomes in the same animal. A careful study proves that this is of common occurrence, regardless of the species concerned.

2. *The Rôle of Chromosome Variations in Heredity.*

Theoretical.—It has been believed for a long time that the chromosomes of the germ cells play the most important part in heredity. Their individuality seems to be established as the result of the work of many investigators. The picture which we now have of each individual germ cell chromosome is a body which is able to transmit a certain group of characters in the formation of a new organism. Each corresponding chromosome in different cells would represent the same group of characters while the different chromosomes of each cell would represent different groups of characters.

We have the right to assume that some chromosomes within the cell have greater influence in determining the characteristics of a new individual, otherwise they would all have equal power in this respect, which is inconceivable. Especially is this so when we see the great difference in the size of chromosomes within the same cell because there must be some purpose in this if the chromosomes are so important.

Naturally the larger chromosomes would represent larger groups of characters or else power to make the characters, which they determine, more prominent.

By a variation then in size of any one chromosome either the number or the intensity of the characters, which it represented, would be varied. If the chromosome is increased, its influence in the determination of the characteristics of the offspring would also be increased.

Knowing that the offspring from the same parents show considerable variation in their characteristics and having found that corresponding chromosomes of the same animal vary in size it seems probable that we have in this the cause of continuous variation.

According to this theory if an immature animal were mated with one of maturity, on account of the smaller size of its germ cell chromosomes (shown in the first portion of this paper) the immature germ cell would be overshadowed by the mature germ cell in the determination of the characteristics of the resulting offspring. In many cases this seems to be true though it is difficult to obtain trustworthy data.

V. Conclusion.

1. Germ cell chromosomes of very young nymphs are smaller than those of adults; this difference bears a direct relationship to the size of the germ cells.

2. As a result of either unequal growth or unequal division, corresponding chromosomes of different cells within the same individual vary in size.

3. The theory suggested by the observations in this paper is that the variation in the size of corresponding chromosomes of germ cells is the cause of continuous variation among animals.[1]

[1] The author is indebted to Dr. C. E. McClung for criticism.

PLATE I.

FIGS. 1 to 24 inclusive. Polar view of chromosomes in first spermatocyte metaphase. Drawings of this and the succeeding plates made with the aid of a camera lucida.

Figs. marked *A* are from adults, *A* and A^1 being from different animals. Figs. marked *n* are from young nymphs. *n*, n^1, n^2, n^3, n^4 being from different animals. Chromosomes are numbered according to their size in the cell beginning with the smallest. The difference in size between nymph and adult cell chromosomes is very striking. A comparison of corresponding chromosomes of different cells of the same animal shows the variation in size mentioned in this paper.

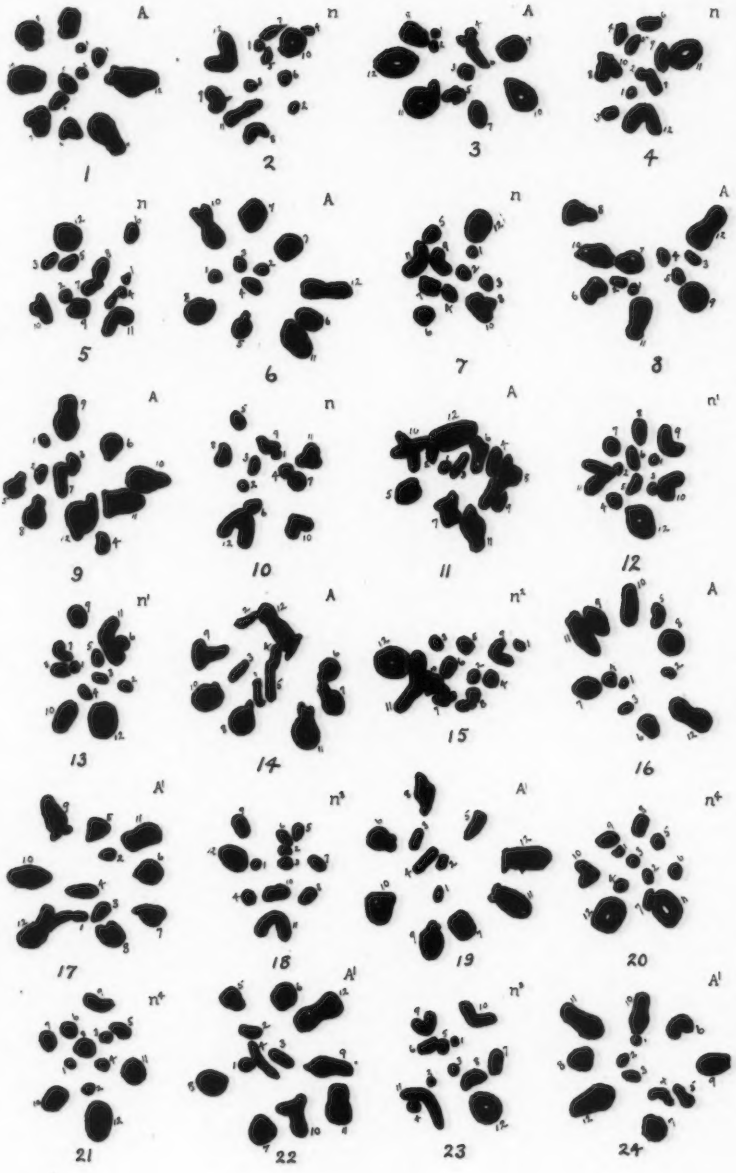
A
n
A
n
1
2
3
4
n
A
n
A
5
6
7
8
A
n
A
n'
9
10
11
12
n'
A
n²
A
13
14
15
16
A'
n³
A'
n⁴
17
18
19
20
n⁴
A'
n³
A'
21
22
23
24

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PLATE II.

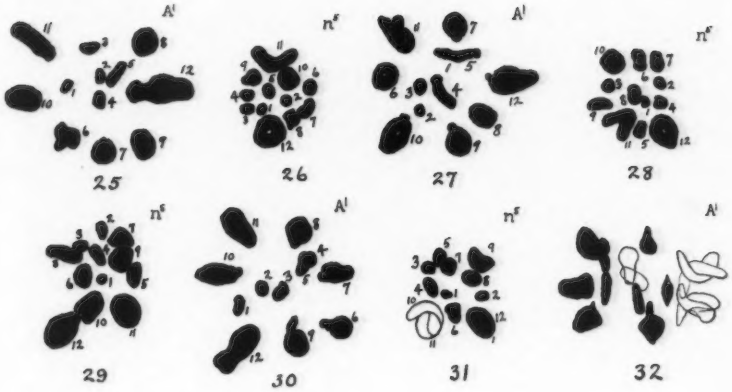
FIGS. 25 to 32 inclusive. Polar views of chromosomes in first spermatocyte metaphase.

FIGS. 33 to 47 inclusive. Polar views of first spermatocyte anaphase.

Scheme of lettering and numbering as in Plate I., n^5 and n^6 being from animals not represented in Plate I.

The greater size of the chromosomes of adults more pronounced than in Plate I.

In both metaphase and anaphase the variation in size, between corresponding chromosomes of the same animal, is evident.


A'
n⁶
A'
n⁶
25
26
27
28
n⁶
A'
n⁶
A'
29
30
31
32

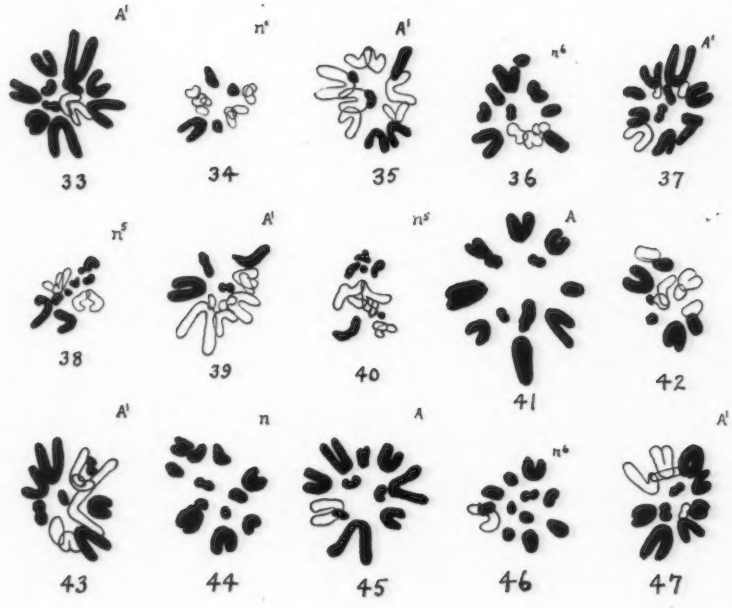

A'
n⁶
A'
n⁶
A'
33
34
35
36
37
n⁶
A'
n⁶
A
38
39
40
41
42
A'
n
A
n⁶
A'
43
44
45
46
47

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PLATE III.

FIGS. 56 to 63 inclusive. Polar views of the first spermatocyte metaphase, showing the variation in size of corresponding chromosomes of the same animal.

Figs. 56 and 57 are from one animal, Figs. 58 and 59 are from one animal, Figs. 60, 61, 62 and 63 are from one animal.

FIGS. 48 to 55 inclusive. Polar views of the second spermatocyte metaphase. Figs. 48, 50, 53 and 55 are from young nymphs, 48, 50 and 55 being from the same animal. Figs. 49, 51, 52 and 54 are from adults, all but Fig. 52 being from the same animal.

The chromosomes and cells of the adults are larger than those of nymphs as in earlier stages. Corresponding chromosomes of the same animal also vary in size at this stage as well as in the next stage (anaphase) represented by Figs. 64 to 67 from the same animal.

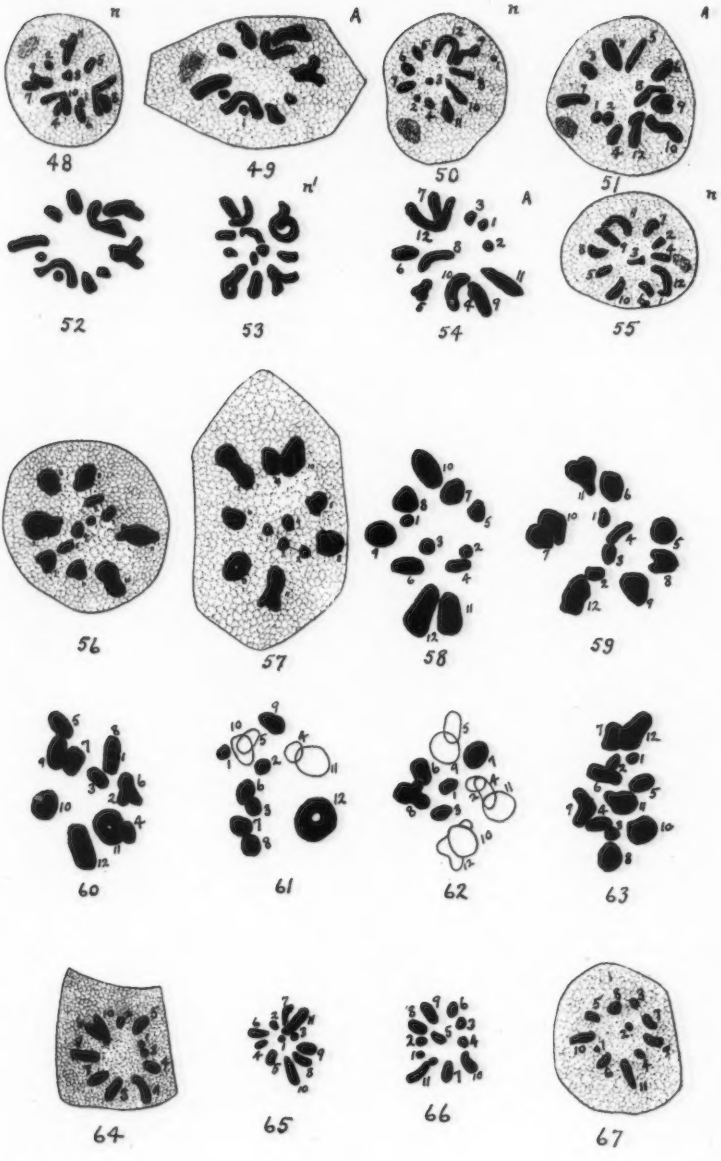

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PLATE IV.

FIGS. 68 to 74. Stages in the development of a single chromosome (*r*) during first spermatocyte prophase. The stain had been removed from all of the other chromosomes except the accessory.

FIGS. 75 to 82. Granules of the corresponding chromosome from other cells of the same animal.

Fig. 75. All granules divided.

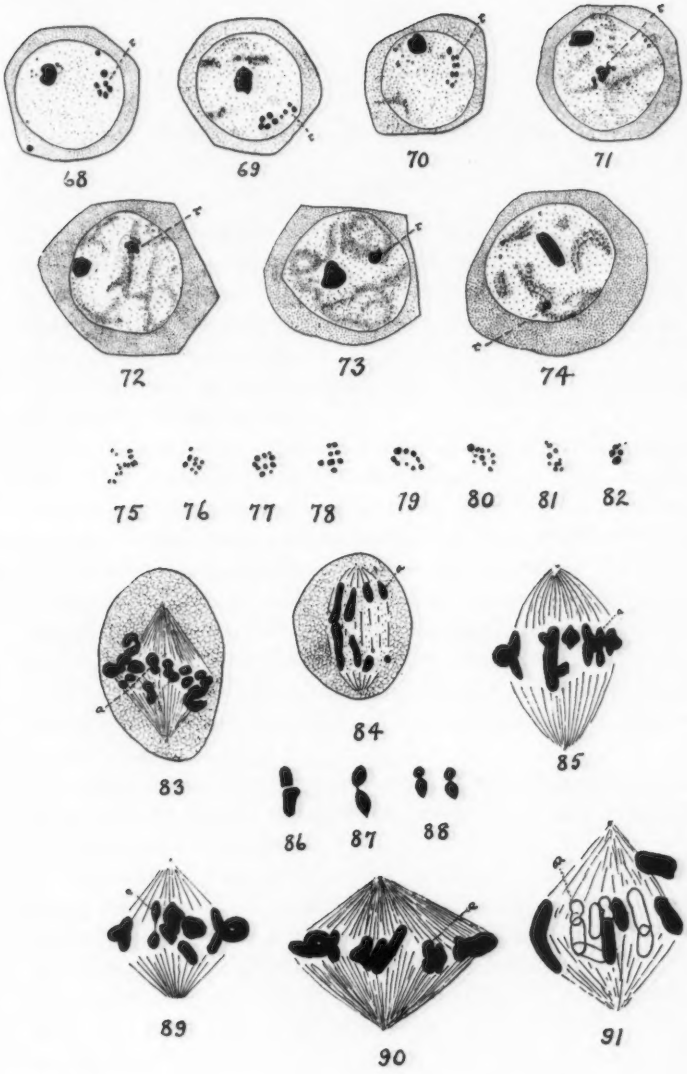
Figs. 71 and 72. Granules in the process of fusion.

The granules vary in size and number at similar stages.

FIGS. 85, 89, 90 and 91. First spermatocytes, showing chromosomes (*a*) dividing unequally.

FIGS. 86, 87 and 88. Other examples from the same stage, those in 88 being from the same cell.

FIGS. 83 and 84. Second spermatocytes, showing chromosomes dividing unequally.



F. R. HARTMAN.

GIANT GERM CELLS IN THE GRASSHOPPER.

FRANK A. HARTMAN.

Giant somatic cells have often been described but so far as the available literature shows nothing but giant spermatids have been figured in the male germ cells of insects.[1] Mitotic figures of giant germ cells throw light upon the development of these abnormal spermatids.

One *Schistocerca* nymph about one third grown contained several giant cells in mitosis.

The earliest stage found was a secondary spermatogonium (Fig. 1) containing forty-six or more chromosomes. The chromosomes were so massed that it was difficult to determine the exact number, but the cell was equivalent to at least two ordinary cells (a typical spermatogonial cell contains twenty-three).

Two giant cells in first spermatocyte metaphase appeared; one of these did not show the chromosomes distinctly while the other (Fig. 2) contained twenty-nine chromosomes. As the number of chromosomes in the spermatocyte stages is reduced one half (to twelve) this giant cell corresponds to at least two normal cells.

An adult *Melanoplus* contained two giant first spermatocytes, each with twenty-four chromosomes, one was in late prophase and the other in metaphase.

A number of giant cells in first spermatocyte anaphase were found in the nymph already mentioned. Of these two are figured (Pl. I., Fig. 3, and Pl. II.). The cells were so large that they were divided into four pieces by sectioning. These pieces were easily distinguished by their relation to the surrounding cells. No doubt a few of the chromosomes were lost in sectioning, as one or two chromosomes were found detached from any cell.

There were fifty-four chromosomes passing toward one pole and forty-nine toward the other in the first of these cells.

[1] Paulmier, F. C., "The Spermatogenesis of *Anasa-tristis*," *Journal of Morphology*, XV.

In the second there were ninety-six on one side and eighty-one on the other. The first was equivalent to four or five cells and the last to eight cells. The chromosomes in these giant cells seem to act normally, that is, they divide and pass to the poles and maintain their individuality in every way, but the cell as a whole fails to divide.

In pathological mitosis the spindles are often multipolar and the number of chromosomes passing to each pole is then very unequal, while in these giant germ cells the mitotic figure is always bipolar and the number of chromosomes passing to each pole is nearly the same.

It may be that giant spermatozoa, formed from such cells as these just described, are the cause of monstrosities when they succeed in fertilizing an egg.

PLATE I.

FIG. 1. A giant secondary spermatogonium containing about forty-six chromosomes.

FIG. 2. An abnormal first spermatocyte with twenty-nine chromosomes.

FIG. 3. Chromosomes from a single first spermatocyte during anaphase. The cell of this figure and the cell represented in the succeeding plate were so large that they were each cut into four pieces in the process of sectioning. Fifty-four chromosomes toward one pole, forty-nine toward the other.

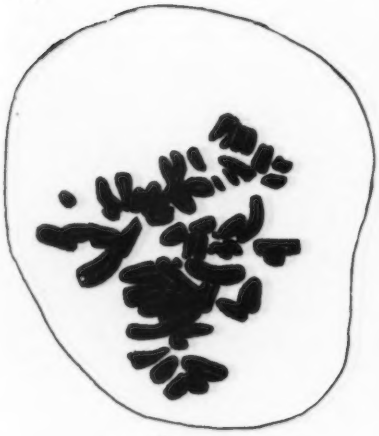


Fig. 1

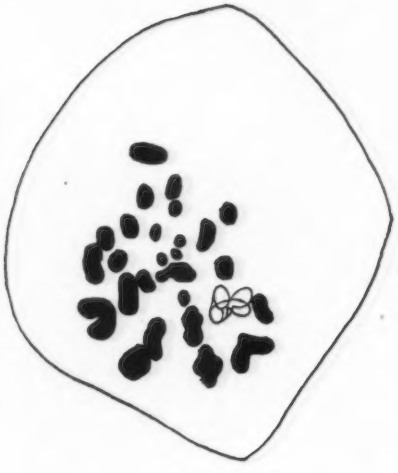


Fig. 2

Fig. 3

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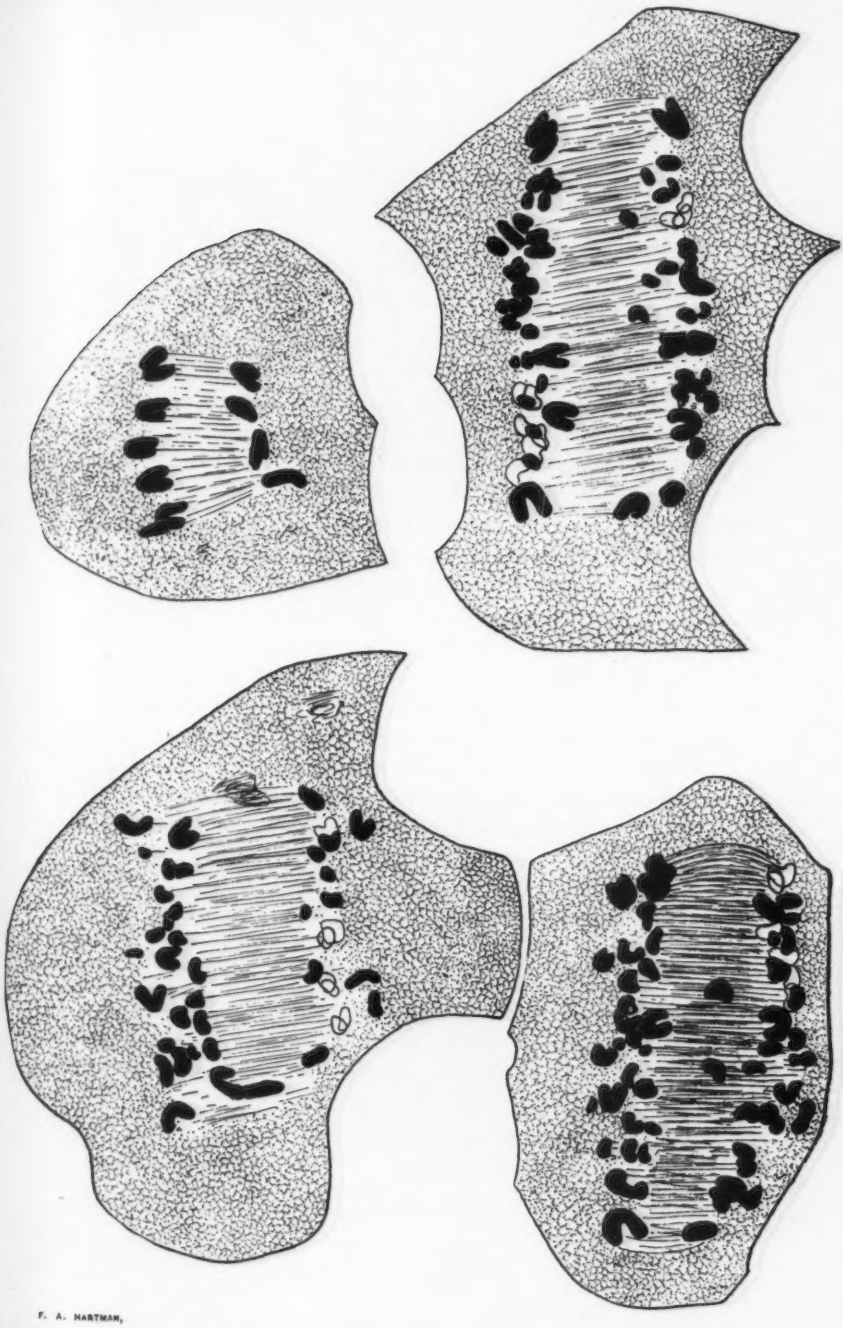
PLATE II.

A giant first spermatocyte in anaphase with ninety-six chromosomes on one side and eighty-one on the other.



F. A. HARTMAN,

THE FERTILITY OF CECROPIA EGGS IN RELATION TO THE MATING PERIOD.

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Nature is lavish in her provision and wasteful in her economy. It is often difficult to tell where her reckless generosity ends and where the delicate limitations for the good of the species begin; in how far the individual shapes the race, or how far natural selection or other agencies eliminate the individuals in unnoticed millions in order to make the species the unit.

The *Cecropia* moth lays, on an average, about 300 eggs. After a free and natural mating, a considerable portion of these are found to be infertile. May it be that the prolific production thus tends to defeat itself by causing the great elimination?

We have also found[1] that a large per cent. of the females die still retaining a considerable number of their eggs, which it seems they have not sufficient strength or lease of life to deposit. This waste might also appear the direct result of the strain of producing such a large mass of ova.

Now we know[2] that the act of mating itself seems to curtail the life of the female by several hours, but even under these conditions of abbreviated time, oviposition is more nearly complete than when the individual remains unfertilized. Hence we see that while copulation itself works against the individual in curtailing its life, it works for the race in, in some way, increasing her power of bringing forth her young. In this far we find copulation, besides being a biological necessity, an actual economy in the production of a next generation.

Now when we consider these phenomena in connection with the fact that on an average 21 hours, or about 9 per cent. of the whole life of the insect, is normally spent in mating, the simple questions at once arise:

[1] *Trans. Acad. Sci. St. Louis*, Vol. 20, p. 314.

[2] *Journ. Exp. Zoöl.*, Vol. 12, p. 199.

Does a longer period of copulation tend to insure the fertility of all the ova? or,

Does a larger number of ova require a longer period of mating in order to insure the fertility of all?

Is there a higher percentage of fertility among the first eggs deposited than among the last? (If so, the eggs retained after death may not be such a waste after all.)

At first thought one might reasonably assume that after a mating of normal duration,[1] practically all of the ova would be fertile. A moment's reflection tells us, however, that this is not the case anywhere in the animal or plant world. But there is nothing to tell us whether a shorter period of mating will result in a lower percentage of fertility. One might reasonably expect that in an abbreviated time the amount of sperm received into the seminal receptacle would be insufficient to fertilize the normal number of ova, and even more inadequate for an abnormally large number.

Whether or not such relationships exist, the following work will tend to show.

Material and Methods.

The 70 insects (35 ♂'s and 35 ♀'s) which supplied the following data were all isolated immediately upon leaving the cocoon, and as fast as mates emerged they were placed together, a single pair in a cage. Thus promiscuous mating was securely safeguarded against. All of the insects were comparatively young when mating began; none were over 24 hours old.

Frequently a few eggs would be deposited before mating began, but these were in all cases vigilantly destroyed. Early every morning the eggs from each cage were counted and placed in a vial with a cotton stopper and labeled; this comprised the oviposition of the preceding 24 hours.

The emerging caterpillars all died in their vials. Later these, as well as the empty egg-shells, were counted, and all the eggs which had not hatched were dissected to find whether they were infertile or whether they contained an embryo. In most cases

[1] Previous investigations have led us to conclude that, in this locality at least, the species is monogamous.

all of the eggs that were shrivelled proved infertile and contained only a dried mass of yellow substance. It also usually happened that the smooth, round eggs contained a full-formed insect. There were a few exceptions in each class, however, and external appearance was not relied upon, but all eggs were dissected. Again a number of fertile eggs had openings of various sizes, sometimes too small to free the insect, and at other times perfectly cut, but the caterpillar it seems felt not inclined to leave the shell. These were in every case counted with those which normally emerged.

Fertility of Eggs and Duration of Mating.

The table shows the fertility of the eggs in relation to the time spent in copulo. All those which continued for about the normal length of time, 16 hours or more, behaved naturally and severed of their own accord; the others (3 to 15 hours) were for the most part artificially separated to ascertain the effect of the shortened period upon the fertility. The table is divided into first day's deposit, second day's deposit, etc., after mating.

An inspection of the columns reveals that there is absolutely no relation between the period of copulation and the fertility of the eggs.[1] We find that the insects which remained in copulo from 3 to 15 hours deposited just as large a per cent. of fertile eggs, and also as large actual numbers, as those which continued from 18 to 48 hours. Hence our present material at least would lead us to conclude that the first three hours are quite sufficient, and that a longer period is only a waste of valuable time and vitality. Especially does this seem distinctly disadvantageous to the species when we consider the number of eggs which the females produce but can not deposit in their short lives. If females which remain in copulo only 3 hours deposit as large a per cent. of fertile eggs as those which continue for 24 hours or more and afterward die without completely ovipositing, it would seem as though natural selection had missed its mark in not shortening the period of copulation and thereby lengthening the chance for oviposition.

[1] The per cents of fertility were also calculated and tabulated for each individual of the entire lot, but these also showed no relationship whatsoever. Since the data are submitted it was thought superfluous to publish these per cents here.

♀ No.	Hours in Copulo.	1st Day's Deposit.				2d Day's Deposit				3d Day's Deposit.				4th Day's Deposit.				5th Day's Deposit.				6th Day's Deposit.				7th Day's Deposit.				Total.			
		T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	Dep. posit.	F.H.	F.U.	Inf.
1	3	166	138	25	3	20	0	20	0	14	1	13	0	13	0	13	0	10	0	6	4	—	—	—	—	—	—	—	—	223	139	77	7
2	3	99	92	1	6	121	97	19	5	45	20	5	20	8	0	7	1	—	—	—	—	—	—	—	—	—	—	—	—	273	209	32	32
3	3	181	148	3	30	128	101	24	3	26	25	1	0	17	8	2	7	3	0	3	0	—	—	—	—	—	—	—	—	355	282	33	40
4	3½	205	169	25	11	83	7	73	3	44	1	0	43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	332	177	98	57
5	4	189	158	2	29	?	?	?	?	?	?	?	?	?	?	?	?	42	30	7	5	6	4	0	2	—	—	—	—	237	192	9	36
6	4	80	62	2	16	96	69	19	8	62	6	4	2	2	?	?	?	1	0	1	0	—	—	—	—	—	—	—	—	239	137	68	34
7	4	140	132	0	8	120	118	1	1	6	4	2	0	?	?	?	1	1	1	0	0	—	—	—	—	—	—	—	—	269	256	4	9
8	5	89	86	0	3	62	3	58	1	27	8	16	3	?	?	?	1	1	0	1	0	—	—	—	—	—	—	—	—	179	97	75	7
9	12	20	14	0	6	68	53	2	13	95	93	1	1	21	18	1	2	10	7	1	2	—	—	—	—	—	—	—	—	214	185	5	24
10	2+8*	24	12	1	11	95	89	0	6	37	28	7	2	82	79	2	1	34	32	2	0	8	8	0	0	—	—	—	—	280	248	12	20
11	12	155	143	1	11	67	60	1	6	11	11	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	233	214	2	17
12	12	94	63	8	23	104	98	1	5	?	?	?	?	151	146	1	4	—	—	—	—	—	—	—	—	—	—	—	—	349	307	10	32
13	12	20	17	1	2	150	42	9	99	4	3	0	1	7	5	1	1	7	5	2	0	51	24	11	16	28	4	2	22	267	100	26	141
14	14	71	71	0	0	26	24	2	0	8	0	0	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	105	95	2	8
15	14	81	57	4	20	255	245	2	8	55	44	4	7	15	6	7	2	3	0	3	0	—	—	—	—	—	—	—	—	409	352	20	37
16	14	12	9	1	2	46	38	6	2	55	50	5	0	47	30	16	1	—	—	—	—	—	—	—	—	—	—	—	—	160	127	28	5
17	14	77	61	8	8	220	206	8	6	82	73	4	5	27	11	7	9	—	—	—	—	—	—	—	—	—	—	—	—	406	351	27	28
18	14	69	33	3	33	130	105	5	20	23	17	5	1	7	3	1	3	—	—	—	—	—	—	—	—	—	—	—	—	229	158	14	57
19	14	166	126	3	37	91	84	3	4	114	110	2	2	4	1	3	0	28	5	23	0	—	—	—	—	—	—	—	—	403	326	34	43
20	15	93	83	3	7	57	43	9	5	114	105	6	3	70	60	3	7	19	2	17	0	4	0	4	0	—	—	—	—	357	293	42	22
21	16	112	11	1	100	110	35	5	70	84	77	7	0	29	21	8	0	—	—	—	—	—	—	—	—	—	—	—	—	335	144	21	170
22	18	140	126	2	12	63	55	4	4	26	17	9	0	12	7	5	0	5	0	5	0	—	—	—	—	—	—	—	—	246	205	25	16
23	24	131	125	5	1	92	68	20	4	30	7	4	19	42	42	0	0	—	—	—	—	—	—	—	—	—	—	—	—	295	242	29	24
24	24	123	0	0	123	41	0	0	41	15	11	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	179	11	2	166
25	24	158	5	17	136	32	20	9	3	27	16	10	1	16	13	2	1	10	5	5	0	—	—	—	—	—	—	—	—	243	59	43	141
26	24	114	77	17	20	82	80	2	0	12	11	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	208	168	19	21
27	24	190	135	51	4	74	40	32	2	31	15	16	0	33	21	9	3	—	—	—	—	—	—	—	—	—	—	—	—	328	211	108	9
28	24	111	0	0	111	121	0	4	117	28	7	0	21	2	1	0	1	—	—	—	—	—	—	—	—	—	—	—	—	262	8	4	250
29	24	56	0	0	56	41	0	0	41	25	0	0	25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	122	0	0	122
30	24	165	5	6	154	48	18	3	27	9	5	2	2	?	?	?	?	3	0	0	3	—	—	—	—	—	—	—	—	225	28	11	186
31	48	77	45	26	6	3	3	0	0	3	3	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	80	48	26	6
32	?	36	18	1	17	3	2	0	1	58	0	0	58	49	0	0	49	47	0	0	47	53	0	0	53	3	0	0	3	249	20	1	228
33	?	240	184	31	25	2	0	2	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	242	184	33	25
34	?	78	55	10	13	81	74	5	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	159	129	15	15
35	?	121	95	10	16	132	102	5	25	49	2	29	18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	302	199	44	59

T. = total. F.H. = fertile eggs that hatched.

F.U. = fertile eggs unhatched. Inf. = infertile eggs.

* In copulo two hours one day and eight hours the next.

Furthermore, it is found that when the percentages of fertility of each day's deposit are calculated, in two thirds of the individuals the fertility increases as the days go by, while in only one third is there a decrease. Hence we are forced to admit another heavy loss; for this evidence would lead us to conclude that a large proportion of the eggs retained after death were also probably perfectly good.

In the material here considered we find also no correlation between this phenomenon and the long or short duration of copulation.

The table shows that in some of the cases of prolonged mating, the first day's deposit of eggs contained a great number which were infertile. Why this should be has not been ascertained. Being confident that all eggs laid previous to mating were destroyed, we are at a loss to explain why the first eggs, which one would think had the best opportunity of being fertilized, were otherwise. But a stranger fact still is that while female 29 was in copulo 24 hours, not one egg was fertile, and almost the same condition existed in females 24, 28 and 30.

One might think that those insects which carried an abnormally large number of eggs would have less chance for their complete fertilization. According to the data in our table, however, no such relation is found to exist. In fact the high percentage of fertility in many of these cases might suggest that the vigorous condition of the female contributed more toward insuring the fertility of the eggs than did the amount of sperm or the duration of copulation.

In all of the eggs in these experiments, then, 77 per cent. were fertile and 23 per cent. infertile. In the next to the last column of the table is given the number which partially developed but, from some unknown cause, died in the shell before hatching. These constitute 14.5 per cent. of the fertile eggs, or 11 per cent. of the whole number deposited after mating. All of the eggs were kept under identical conditions, so if 85.5 per cent. of the fertile ones hatched, the fact that these 14.5 per cent. did not hatch can not be attributed to environmental conditions.

Some of the eggs are laid in closely adhering clusters, and some are dropped singly. It was suspected for a time that the cater-

pillars had more difficulty in emerging from the clustered eggs and that many died in the attempt, but it was soon found that as many died in the shell in the loose as in the adhering eggs.

Hence the proportion which go to the wall even up to the time of emerging is large. We are reminded of Darwin's[1] statement that the ". . . geometrical tendency to increase must be checked by destruction at some period of life . . . and this period in the great majority of cases is an early one. . . . But if many eggs or young are destroyed, many must be produced or the species will become extinct." In our present material it would seem that nature, in making the attempt at providing so bountifully for the perpetuation of the species, has overstepped the limits of real economy by wasting so much unfinished material.

[1] "Origin of Species," 6th ed., p. 52, 1887.

THE LIFE HISTORY OF DESMOGNATHUS FUSCA.

INEZ WHIPPLE WILDER.

SMITH COLLEGE.

Although *Desmognathus fusca* ranks as one of the commonest of the American salamanders, at least in the eastern part of the United States, our published accounts of its life history are singularly meager and fragmentary. It is hoped, therefore, that the following account, culled from observations extending through several years during which this species has served as material for class work in anatomy, histology, embryology, and physiology in our laboratory courses at Smith College, as well as for several lines of individual research, may prove of some interest and even practical value to others who are engaged in the study of amphibian life. Incidentally, too, there is always the possibility that facts presented for their intrinsic interest may prove to have some broader biological significance, particularly in a field like that presented by the amphibians, in which there are involved, both phylogenetically and ontogenetically, so many environmental transitions which call for adaptive response.

SYSTEMATIC POSITION AND SPECIFIC CHARACTERS.

Desmognathus fusca is a representative of the family Desmognathidæ, which, together with the family Plethodontidæ comprises all of the American Urodeles now known to be lungless.[1] In common with the other members of these two families, *Desmognathus* possesses the naso-labial groove (Whipple, '06*a*) and lacks the ypsiloid cartilage (Whipple, '06*b*).

The following description of the external appearance of *Desmognathus fusca* is given by Morse ('04, p. 115): "*Desmognathus fusca fusca* Raf. Above dark, with a median dorsal band of lighter generally brown in color and specked with black. Below gray with black specks forming a marbling. . . . Very variable in color. Costal grooves 14. Length 4½ inches."

[1] *Amblystoma opacum*, reported lungless by Lönnberg, has since been shown to possess fully developed, functional lungs (Whipple, '06*b*).

As pointed out by H. H. Wilder ('99), *Desmognathus fusca* may be conveniently distinguished from *Spelerpes bilineatus*, the other lungless species with which it is usually associated in habitat, by its more robust body, its general darker color, and less distinct markings. In *Desmognathus* the markings become more and more obscure with the increasing size and darkening color of the older adults. In many even of the smaller adult *Desmognathus*

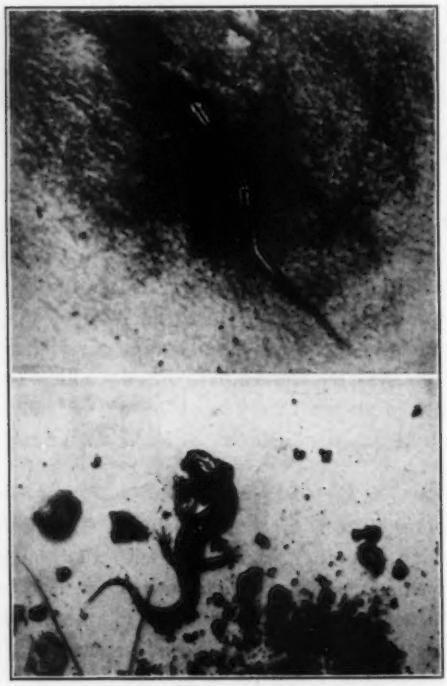


FIG. 1. Photographs of living adult *Desmognathus fusca*, in two characteristic attitudes, about one half natural size.

individuals the whole color becomes very dark and the ventral surface thickly mottled with pigment spots, while *Spelerpes bilineatus* remains practically unpigmented ventrally. The larvæ of the two species are, however, even more frequently confounded. Their differences have been clearly shown by

Wilder ('99). *Desmognathus fusca* larvæ undergo metamorphosis while still very small, from 28 to 30 mm. in length (although the duration of larval life is much longer than Wilder's estimate), while *Spelerpes bilineatus* may attain before metamorphosis a length of over 50 mm., greater indeed than the length of certain adult specimens. The *Desmognathus* larvæ may be distinguished from the younger *Spelerpes* larvæ by the proportionate size of the hind legs, which in *Desmognathus* are from the time of hatching much larger and stouter than the fore legs and are held when at rest at a considerable angle from the body, while the hind legs of the small *Spelerpes* larvæ are slender and scarcely exceed the fore legs in length. The digits of both fore and hind feet of *Spelerpes* larvæ of all sizes are attenuated, and the feet never have the robust appearance of those of *Desmognathus* larvæ.

Morse ('04) says of *Desmognathus fusca:* "The larvæ attain a length of three inches before becoming mature, and inhabit springs and small bodies of water. They are brown with black dots above, lighter below, retaining also the black dotting. The gills are short and inconspicuous. Sometimes the black is dotted here and there with livid specks of red, but this disappears in the adult condition." This is undoubtedly a case of incorrect identification as the description does not correspond to *Desmognathus fusca* larvæ in the matter of size, color, or habitat. I am able to refute the identification with considerable positiveness for the reason that my conclusions concerning every stage of the life history of this species have been corroborated by careful study for several successive years of material obtained from a much circumscribed little stream in which *Desmognathus fusca* is the only salamander that occurs.

Habitat.

As compared with other Urodeles, *Desmognathus fusca* may well rank as one of the more terrestrial. Its lungless condition is in itself an indication of its terrestrial habitat; for, as pointed out in a previous paper (Whipple, '06*b*), amphibian lungs serve an important hydrostatic function, so that with the exception of certain lungless forms such as *Spelerpes ruber*, which have

secondarily adapted themselves perfectly to aquatic life at the bottom of the water, the lungless condition unfits an amphibian for a prolonged stay in the water. If further evidence on this point is desired one has only to drop living specimens of *Diemyctylus viridescens* and *Desmognathus fusca* into a deep aquarium and compare their behavior!

The lungless salamanders show, however, varying grades of environmental adaptation, from *Spelerpes ruber* above cited as a perfectly aquatic form, to *Plethodon cinereus*, which is completely

FIG. 2. Photograph of a typical habitat of *Desmognathus fusca*, as it appears in late autumn, when the water in the little brook is for the most part covered with fallen leaves, which serve as a protection to both the larvæ and the adults.

terrestrial even during its larval life, while the extreme is reached in the case of *Autodax lugubris*, reported by Ritter and Miller ('99) to be practically arboreal throughout its entire life.

Desmognathus fusca is terrestrial to the extent that it lives in and upon moist earth, where it mates and lays its eggs, and

where the first few days of the larval life are spent. The young larvæ, however, soon reach shallow running water where they remain for nearly a year, after which they come out of the water to live the terrestrial adult life of the species. The presence of aquatic as well as terrestrial insect forms in the stomachs of adult *Desmognathus* shows, however, that even the adults are by no means absolutely terrestrial, but, so far as my own observations and those reported by others go, the adults confine their aquatic excursions to very shallow water, and they are seldom found in the water at all unless driven there.

Apparently, the ideal environmental conditions for *Desmognathus fusca*, as deduced from a study of those localities in which they occur in the greatest abundance, are those afforded by the banks of well shaded streams of shallow, perennially running water. In such places they may usually be found under every stick and stone and fallen log at or below the water level, while the soft earth is riddled with burrows, often, it is true, the work of earthworms or of other animals, but utilized as convenient lurking places for *Desmognathus;* for the adult *Desmognathus* is, in the day time, at least, thigmotropic, and will even take refuge in a bottle left lying on the surface of the soil exposed to the light, usually orienting the body with the head toward the mouth of the bottle. *Desmognathus* has the power to make its own burrows, as it will demonstrate, sooner or later, when isolated in a terrarium which affords no other suitable means of protection. In one case in particular which came under my observation, two individuals were accidentally set aside in a terrarium which was supposed to contain no specimens. Over a year afterwards it was found that these animals were still living in this terrarium under almost perfectly dry conditions. In the soil were many well formed burrows, within which the specimens, which were kept for some time after they were discovered, remained, often completely concealed, but at times with the head protruding from the entrance to the burrow.

Under natural conditions the crevices and burrows inhabited by *Desmognathus* are, of course, very moist, and I have usually been able to demonstrate, by following them carefully through their windings and branchings, a connection with the water of then eighboring brook.

The part which the shaded condition plays in the habitat of *Desmognathus* is undoubtedly a somewhat complicated one, possibly determining conditions for the necessary food supply, as well as helping to maintain a moist condition of the soil, supplying an abundant and annually replenished loose earth from fallen leaves and twigs; those leaves, also, which fall into the water furnish lurking places in which the larvæ take refuge and seek their food. The element of close proximity to running water in the habitat of *Desmognathus* is certainly not necessary to the immediate physiological demands of the adult, but is incident rather to the aquatic nature of the larval life, which makes necessary not only easy access to water after hatching, but also requires that the supply of water shall be perennial, since each year the newly hatched larvæ reach the water at about the time when the brood of the previous summer leave the water as very small adults. Upon the other hand, these small adults upon leaving the water find themselves in the midst of the proper external conditions for their whole adult life, including mating and egg laying, and therefore, so far as is known, there is no tendency whatever to annual migrations such as has been shown to occur in *Amblystoma punctatum*, for example (Wright, '08).

Mating Habits.

In his valuable discussion of the breeding habits of amphibians, Smith ('07) has pointed out an interesting gradation in methods of fertilization from the lavish method of typical aquatic fertilization possessed by *Cryptobranchus*, in which the unfertilized eggs are expelled into the water in large numbers, to take their chances at being fertilized by the abundant spermatic fluid expelled by the male in their vicinity, to the opposite extreme of typical internal fertilization without spermatophores, which the *Apoda* have been shown to have acquired as an adaptation to their completely terrestrial existence. Between these two extremes lie such forms of fertilization as that of the *Amblystoma punctatum*, in which the spermatozoa are not poured freely into the water, but are enclosed within spermatophores which the male, stimulated by the presence of the ripe or nearly ripe female, deposits in considerable numbers, leaving with the

female, however, the whole responsibility for the entrance of one of these into her cloaca (Wright and Allen, '09); and the still more certain method of the various *Tritons*, in which the male uses special devices, such as clasping, to ensure the entrance into the cloaca of one of the small number of spermatophores, thus making certain the fertilization of a far larger proportion of the reduced number of eggs. Smith ('07) points out that in the increasing economy in the amount of seminal fluid shown by such mating habits as those of the *Amblystoma* and *Triton* we see, as an incidental result, a preparation for possible terrestrial life.

In *Desmognathus fusca* we see a form which has availed itself of this, as well as of other possibilities of adaptation to terrestrial existence; for, although the larvæ retain the typical aquatic nature of amphibians, the habit of a periodical return to the water for the mating and egg laying which an aquatic larval life usually involves has been abandoned, and both of these functions are performed by the adults without the inconvenience of leaving their terrestrial abode. My information concerning the actual act of mating is drawn from a single observation. Since, however, both Kingsbury ('02) and Hilton ('09) state that nothing definite seems to have been published upon this point, I feel warranted in giving a full report of this single observation.

On the evening of May 13, 1908, I isolated in a small terrarium a large male, and a female through the abdominal wall of which large eggs could be seen. It was discovered the next morning, however, that another smaller male was also present in the terrarium, probably having been carried over unobserved in transferring some wet leaves. On the following morning, May 14, the female and this smaller male were found lying upon the earth under some wet leaves, the ventral surfaces of the bodies in contact. They reacted so quickly, however, to the disturbance of the leaves that beyond this very hasty observation as to their general position I can state nothing definite as to methods of clasping or exact regions of contact. Protruding from the cloaca of the female was a yellowish, semi-fluid mass which was found upon examination to be a spermatophore of very soft consistency.

When placed in a drop of water upon a slide, the spermatophore

was found to contain large numbers of tightly coiled spermatozoa (Fig. 3, *x*), which, however, in a few minutes began to uncoil, so that in the course of half an hour the majority were uncoiled or uncoiling. Some few, however, were still coiled seven hours later. From time to time some of the spermatozoa were seen to be coiling up again, a condition which was probably induced, as was shown by a subsequent study of spermatozoa taken from

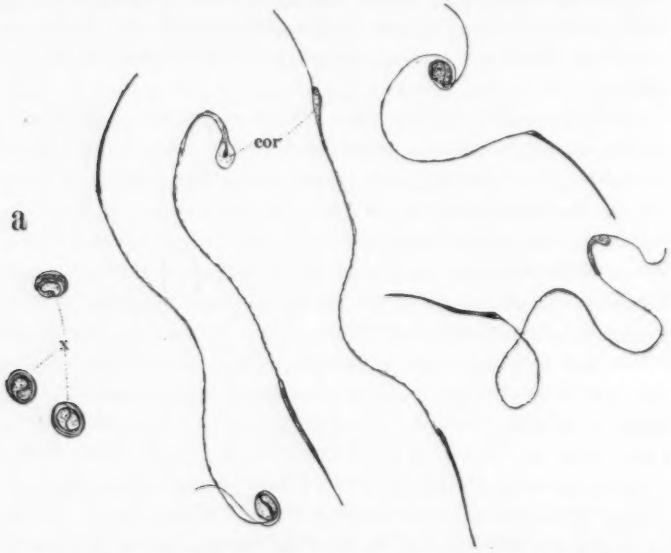


FIG. 3. Spermatozoa of *Desmognathus fusca*, showing method of coiling; *cor*, the mass or corpuscle about which the spermatozoa coil; *x*, spermatozoa coiled as found in the spermatophore. Based upon drawings with Abbé camera. × 290.

the ducts of living males, by partial drying of the preparation. All of these movements were somewhat jerky and mechanical, and none of the free swimming movements such as the living spermatozoa exibit within the ducts of the male were to be seen. It must be noted, however, that the conditions were not perfectly normal, since the spermatophore, obviously not intended for an aquatic medium, was placed in water for examination. In the case of another spermatophore, found in the cloaca of a similarly isolated female, and examined microscopically without the

application of water, the spermatozoa were coiled and exibited none of the uncoiling movements. In this case the spermatophore was of a much firmer consistency, which, as no male was found with the female, may have been due to the longer lapse of time after its transfer to the cloaca of the female.

Kingsbury ('02) concluded from the presence of spermatozoa in the cloaca of the females captured at all seasons of the year, that the fertilization is internal, and further inferred from the condition of the ducts and cloaca of the male at various seasons, that the fertilization takes place not only in the spring but possibly also in the fall. The whole subject is one which demands a careful series of observations and experiments, which are, however, rendered somewhat difficult, as pointed out by Kingsbury, by the retired nocturnal habits of the animal. The above report is at least sufficient to show that the mating in this species takes place under terrestrial conditions and that the fertilization is an internal one, facts which are quite in accord with the general adaptation to terrestrial life shown by *Desmognathus*.

In my examination of spermatozoa I have invariably noted a certain appearance of which I have as yet found no mention in the literature upon the subject. Spermatozoa taken in a living and active condition from the ductus deferentia of a decapitated male, as well as those removed from the cloaca of the female, may be seen to constantly coil and uncoil, assuming in the process such appearances as are shown in Fig. 3. In uncoiling, however, there is gradually revealed an elongated or oval mass (*cor*), apparently of semifluid consistency and of a translucent material, which seems to form the core about which the coiling takes place and which is enclosed in the last final loop of the tail as it uncoils. As spermatozoa within the tubules of the testis do not possess this structure, it is apparently acquired in the passage from the testis into the duct, and is very likely a secretion of mucous nature derived from certain specialized cells. Its nature and source, however, as well as its function require further investigation. If it is peculiar to *Desmognathus*, it may well be an adaptation to the prolonged period which must often elapse between the introduction of the spermatozoa into the spermathecæ of the female and the actual fertilization of the eggs,

and is one of the many devices which it is necessary for a terrestrial form to develop to resist the drying action of the air.

Egg Laying Habits.

Concerning the egg laying habits of *Desmognathus fusca* we have several published reports. According to Hilton ('09), the eggs are found most abundantly from the last of June to the middle or last of July. Kingsbury ('02) reports eggs of *Desmognathus* found at Ithaca during July and August, and refers to the statement of Sherwood ('95) that they have been found from July to October. Reed and Wright ('09) give July as the time of maximum egg laying. In western Massachusetts, in the immediate vicinity of Northampton I have found eggs as early as the eleventh of June and as late as the twenty-fourth of September, although, since in the latter instance the eggs were just at the point of hatching, they were probably deposited during the latter part of August. As reported by H. H. Wilder ('04), eggs have been deposited by individuals in captivity in the Smith College laboratory as early as June first. It is thus safe to state that the egg-laying period extends at least from the first of June to the last of August and possibly through September. As suggested by Hilton, temperature and humidity may have much to do with determining the exact time, and it should be further noted in this connection that the latest brood of my own observations, that of September 24, 1907, occurred at the end of a singularly cold summer, and may have been belated from that cause.

The eggs are laid in a small batch consisting usually of two masses and numbering about 20 in all (15–20 according to Hilton), a number which corresponds in general also to the count of the ripe eggs found in the ovaries of a large number of females which I have examined for purposes of comparison. The total number is practically constant, being 10 or 11 in each ovary. A few show a smaller number or a greater inequality, such as the case of 9 in the left and 5 in the right ovary, or of 13 in the left and 4 in the right ovary.

The small number of eggs deposited at a time as compared, for example, with several hundred reported for *Cryptobranchus allegheniensis* (Smith, '06, '07 and '12), 130–225 for *Amblystoma*

punctatum (Wright and Allen, '09), 108 for *Diemyctylus viridescens* (Jordan, '91), and 150 for *Amphiuma means* (Hay, '88 and '90), shows a striking correlation with the far greater certainty of fertilization which is insured by the direct deposit of the spermatophore in the cloaca of the female; the small number of eggs is also undoubtedly correlated with the superior chances for successful development afforded by the large size of the egg (about 3.5 mm. in diameter), and the maternal care during development.

The eggs are laid in a little moist hollow or cavity, often of accidental occurrence, in just such places, in fact, as those in which the adult *Desmognathus* is usually found. I have observed that the female in captivity will sometimes excavate in the soft earth underneath a stone or clump of moss a cavity sufficiently large to accommodate her body in a coiled position, and in this cavity will deposit her eggs. In nature I have found eggs under a mere covering of moss or a little decaying stick. Hilton ('09) reports that they are found also at a depth of as much as four feet below the surface. They seem never to be deposited more than two or three feet from the edge of the water, or more than a few inches above its level in the neighboring brook, into which the newly hatched larvæ are destined to find their way.

So far as my own observations and those reported by others go, the eggs are laid during the night or in the early morning, as would indeed be expected from the nocturnal habits of the species. No cases have been reported of the prolongation of the process of egg laying beyond the single night during which, apparently, all the eggs produced by a given female for one season are deposited.

The eggs are always found guarded by a female, undoubtedly the mother. She usually so places herself among them as to bring practically all of the eggs in contact with her body, which often extends through the mass of eggs and is frequently bent sharply upon itself as if the better to surround and protect them. When under observation, as in a terrarium, the mother frequently leaves the eggs when disturbed, always retreating through the same exit from the nest. After having been separated from the eggs, however, as may occur in making a transfer from out of

doors to the laboratory, the mother goes back to them again, even though the nest and all of its surroundings may have been reconstructed. I have never had the opportunity to further test the sense of ownership of eggs in a mother by exchanging the eggs of two individuals, but the experiment would certainly be an interesting one.

The function of the mother in incubating the eggs is probably mainly that of insuring the proper degree of moisture, since eggs when removed from the mother and kept moist undergo normal development. It is possible, also, that she may guard them from inundation; for the first eight or ten days eggs will develop normally, even though quite immersed in water, but during the later development a short immersion in water kills them, apparently through a rapid change in osmotic pressure between the cells of the embryo and the fluid which by this time surrounds the embryo within the protective envelopes.

I have no evidence that the habit of eating the eggs, such as Smith ('07) found in the case of *Cryptobranchus*, is ever indulged in by *Desmognathus*, although, as will be shown below, this species is by no means to be exempted from the charge of cannibalism.

The Eggs.

The egg membranes, already very carefully described by previous writers, are three in number, the outer one being continued into a stalk which connects the eggs with the others, and is thus a common membrane for the whole mass. Smith, ('12) in comparing the egg strings of *Cryptobranchus allegheniensis* with the arrangement of the eggs of other Urodeles, suggests the probability that the most closely related egg arrangement is such as is shown by the stalked egg enclosures of *Desmognathus* rather than in the jelly-enclosed masses of certain other forms. The accompanying drawings (Fig. 4) of the two egg masses of a batch of eggs studied by me suggest the probability of the derivation of their arrangement from a more primitive rosary arrangement, such as *Cryptobranchus* possesses. A few eggs are still seen in the main axis of the bunch, but the majority have pushed out to one side, as if through an excess of lateral pressure brought to bear upon the outer envelope from within. Each

egg has thus acquired a short stalk which connects it with the other eggs of the bunch. Whether this is a change which takes place in each individual case during the descent of the eggs through the oviduct and the subsequent reaction upon exposure to air, or whether this is a form of egg string which has been derived phylogenetically from some ancestor in which the egg string was in the rosary form, I am unable to say. The stalk of each egg becomes eventually very much twisted, probably a secondary condition brought about by the movements of the

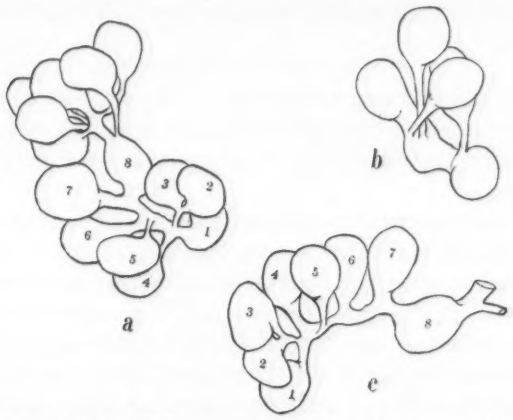


FIG. 4. Sketches of egg capsules of *Desmognathus fusca* after the larvæ have hatched; (*a*) and (*b*), the two masses of the same batch of eggs as is shown in Fig. 6; (*c*) a view of the egg capsules numbered 1–8 of (*a*), so arranged as to show their probable sequence with relation to the main stalk of the mass, and suggesting the derivation of the form of egg mass from a more primitive rosary form. Approximately twice life size.

mother among the eggs while brooding them; this twisting tends to increase the length and diminish the diameter of the stalks, thus crowding the points of their mutual attachment nearer together and emphasizing the relationship expressed by H. H. Wilder's comparison of the whole mass of eggs to a bunch of toy balloons.

The eggs themselves are creamy white, unpigmented spheres, each measuring from 3.5 to 4 mm. in diameter. This large size provides, of course, for a more advanced stage of development at the time of hatching and thus insures the preservation of a

very large percentage of the brood. The large size of the eggs, the certainty of fertilization which the internal method insures, the well-sheltered position in which the eggs are placed, and the maternal care during development are thus all conditions which compensate for the very small number of eggs.

Within the protective envelopes, the eggs orient themselves, after the manner of amphibian eggs, with the animal pole above· they continue this orientation until the embryos reach a sufficiently active stage to introduce other factors into the determination of their position in the egg.

Embryonal Development.

Without attempting to enter into the details of the embryology of *Desmognathus*, certain general features of the course of development of the egg are in place in an account of the life history of the species.

The early development of *Desmognathus* eggs has been worked out by both H. H. Wilder ('04) and Hilton ('04 and '09). The segmentation is found to be holoblastic, although, as demonstrated by Hilton, the large size of the yolk mass prevents the complete division internally until after the early blastula stage is reached, thus placing the egg on the border line between the holoblastic and meroblastic types, while throughout its entire development the appearance of the embryo as it lies at first upon, and later coiled around, the large mass of yolk cells, so strongly suggests the condition found in meroblastic eggs that one instinctively speaks of "embryo and yolk" (Fig. 5). This resemblance is emphasized by the fact that the conspicuous capillary network which early develops on the surface of the mass of yolk strongly suggests the yolk circulation of meroblastic forms. Wilder ('04) noted the correspondence externally of certain of these blood vessels with the lateral cutaneous and median abdominal veins of the adult, and on this ground did not consider them the homologues of the vitelline veins of meroblastic forms. Piersol ('09), on the other hand, describes a similar meshwork of blood vessels in the splanchnic mesoblast of the embryo of *Plethodon cinereus erythronotus* as a vitelline circulation, and as sections of *Desmognathus* embryos show the blood vessels in question to be

similarly situated in the splanchnopleure (Fig. 11, *vbv*), there would seem to be no reason why they should not be considered the homologues of a true vitelline circulation.

The segmentation process results in the distribution of the yolk granules to the cells of the blastula, and there early appears, according to Hilton ('09), a differentiation of the cells into those containing large and those containing small yolk granules, the latter arranging themselves in a superficial layer surrounding the former, which form the whole central portion of the mass. Wilder ('04) found that the late blastula stage is reached in

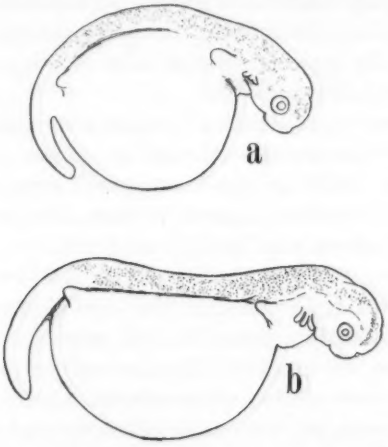


FIG. 5. Two embryos of *Desmognathus fusca* removed from two of the same batch of eggs after 16 days of development, showing a difference in the rate of development. Drawn with Abbé camera. × 9.

about three days, while Hilton reports the formation of the yolk plug stage in about forty hours from the time the eggs are laid. This discrepancy may, however, be due to an individual difference in the rate of development, since Wilder found existing simultaneously in the same batch of eggs, four-, eight-, and sixteen-cell stages.

By the eleventh day both the anterior and the posterior ends of the embryo are well lifted above the surface of the yolk, and the dorsal region of the body appears as a narrow welt or ridge stretching between them. By the thirteenth or fourteenth day

the embryo begins to make spontaneous movements, forcibly bending the free anterior and posterior ends of the body to right and left. By this time the limb buds have appeared, and the dorsal surface of the embryo assumes a decidedly grayish hue, owing to the beginning of pigmentation, which rapidly increases as development goes on, and takes the form of a decided pattern by the twenty-fourth day.

External gill bushes may be readily distinguished as early as the sixteenth day. On the twenty-fifth day, however, neither the mouth nor the gill slits have opened to the exterior. On the thirtieth day the mouth has opened by two lateral slits but is still closed in the middle region. At this time, also, the two most anterior gill slits upon each side are open, the more anterior being just posterior to the gular fold.

A considerable accumulation of liquid early appears about the embryo, and continues to increase in amount, distending the egg envelopes, which are thus placed under a constantly increasing tension. From this cause it becomes increasingly easier to remove the embryo from the egg membranes, until by the eighteenth day a puncture of the membranes is followed by a rapid expulsion of the embryo through the force of the escaping fluid. This is undoubtedly a preparation for the easy rupture of the membranes and escape of the embryo when the time for hatching arrives, the fluid serving meanwhile to equalize the external pressure upon the delicate embryonal tissues and prevent injury to them.

Thirty days is the longest time for which the continuous development of any batch of eggs has been reported (H. H. Wilder, '99), so that as to the exact duration of the period of development within the egg I have very limited data. It is seldom that one knows the exact date when a given batch of eggs is deposited, or, knowing this date, can carry on the observations successfully under artificial conditions to the time of hatching. Hilton states that the rapidity of development depends upon humidity and temperature, but gives no data whatever concerning the duration of the whole period. Wilder describes the thirty-day embryo above referred to as a fully developed larva in all respects except the still large yolk mass. "The pigmentation was com-

plete, the external gills fully developed, and the feet had the full number of distinct toes (4 anteriorly and 5 posteriorly)." This embryo was 13 mm. in length, 2 mm. shorter than the shortest newly hatched larva which I have measured, and Wilder estimated that its supply of yolk might indicate a continuation of development within the egg for several days longer. Thus the length of the entire period might be estimated at approximately 5 weeks.

The length of the period of development within the egg varies, however, even in the same batch of eggs, since the hatching process sometimes continues for four or five days. This difference in the time of hatching may possibly be due to the element of chance in the occurrence of sufficient friction to rupture the membranes; since, however, the newly hatched larvæ have, so far as my observations go, reached the same stage of development, though differing slightly in size, it is more probable that this difference in the date of hatching of members of the same brood is due to an actual difference in the rate of development. The difference which Wilder found in the segmentation stages of eggs of the same brood, which might be logically accounted for by a slight difference in the exact moment of fertilization, would in itself be insufficient to account for so great a difference as four or five days in the time of hatching, or for the decided difference which I have observed in the development of embryos taken simultaneously from the same batch of eggs during the latter part of the embryonal period (Fig. 5, *a* and *b*). Piersol ('09) noted a similar difference in the rate of development and consequent time of hatching in the eggs of *Plethodon cinereus erythronotus*, and attributed it to a difference in the accessibility to a supply of oxygen, the eggs on the outside of the mass having the better chance. In the case of *Desmognathus*, however, the eggs are so arranged and so frequently shifted in relative position by the movements of the mother, that it is hardly possible that they differ greatly in their access to either moisture or oxygen supply. Hilton noticed that the smaller eggs of the mass usually develop more rapidly, a condition which might readily result during the early stages from the smaller mass of inert yolk material upon which to expend the energy of cell division, and possibly

in the later stages from the larger ratio of surface for absorption, to mass.

Hatching, and the Terrestrial Larval Period.

Since the development and hatching of the egg take place under terrestrial conditions, it follows that there must be a terrestrial larval period between the time of hatching and the time when the larvæ reach the water and enter upon their aquatic larval life. Concerning this terrestrial larval life of *Desmognathus* and the important changes which the transition from terrestrial to aquatic conditions naturally involves, there is, so far as I know, no published account. For this reason I shall give somewhat in detail an account of a case of the hatching and subsequent development of a brood of *Desmognathus* larvæ which came under my observation.

The batch of eggs in question were found on September 24. They were found in the usual sort of location, under a decaying stick about three feet from the edge of the water of a certain shallow brook in which *Desmognathus* abounds. A large female was, as is usually the case, coiled about the eggs, while nearby under the same stick was a smaller adult, which proved to be a male. As it is not uncommon to find two or more individuals under the same stick or stone, this fact has probably no significance. The eggs contained large and very active embryos, each still distended with a conspicuous mass of whitish yolk.

As I had not the implements at hand for removing a sufficient mass of the loose earth underneath the nest to avoid disturbing the eggs, I carefully replaced the stick and left the eggs and the adult undisturbed, intending to return immediately and remove them. This a heavy shower prevented, and the nest was not revisited until the next morning. The female was found under the stick two or three inches from the eggs, which had already begun to hatch. She was in a rather poorly defined burrow and was headed away from the eggs. The whole family (mother with the hatched larvæ and unhatched eggs) was taken up upon a mass of soil included in a radius of about five inches and several inches deep, and was carefully transferred to the laboratory. The mother made no attempt to escape and during the journey

returned to the eggs and placed herself among them (Fig. 6). Three larvæ and fifteen unhatched eggs were counted at this time; it is probable that two larvæ had already escaped from the nest, however, as a subsequent examination of the egg envelopes after all had hatched showed the entire number of eggs to have been twenty. The larvæ were very active and crawled about over the moist body of the mother; when disturbed they made quick jerky jumps among the loose debris of pine needles and decaying leaves upon which the eggs were lying. Two of the larvæ were killed at this time and were found to measure 15 and 15.5 mm. respectively (stage *A* of the subsequent description).

FIG. 6. Sketch of an adult female *Desmognathus fusca* with batch of hatching eggs. Drawn Sept. 25, 1907, by H. H. Wilder. Approximately life size.

The whole mass of soil was placed undisturbed in a small round glass terrarium, and the eggs, the remaining larva, and the adult were covered with some large wet leaves. A shallow crystallizing dish, filled with water and buried to the brim in the dirt at the bottom of the terrarium, was provided to take the place of the brook into which, if left undisturbed out of doors, the larvæ would presumably have found their way. Water was added to make the soil as thoroughly moist as in the natural habitat.

For five days the hatching continued at the rate of two or three a day. During this time the behavior of the mother and the young was frequently observed. In the course of the first 24 hours the position of the egg mass was entirely changed, probably owing to the gradual shifting due to the movements of the mother. After two days the mother no longer remained in contact with the eggs, but she stayed nearby in a burrow which she had made in the loose soil; she was usually found lying in

this with her head very near the hatching eggs. Little by little the newly hatched larvæ would stray from the nest, and would be seen, often in groups of two or three, disappearing into deeper and moister crevices in the loose soil. Often one or more would be seen in contact with the mother in her burrow. During the last two days of the hatching period, the mother was still deeper in her burrow, and although she may have returned to her nest unobserved, she never was found there. The larvæ, also, were vanishing, so that by the time the last of them hatched, only eight were visible in the nest.

Later, on October 1, search was made to learn what had become of the larvæ. Several were found in the loose mass of decaying leaves, in burrows which had been made by the mother, or in natural crevices in the loose soil. These were in groups of from two to four with the bodies in close contact. None were in the water in the glass dish; but upon lifting this, two were found in a little pool beneath it, a position which they had evidently reached by going down from the nest into the deeper and wetter layers of the loose debris until they reached the wet surface of the more solid layer at the bottom. In another little pool two others were found. These larvæ, like those in the loose soil above, were very active when disturbed. They placed themselves, as do the aquatic larvæ in their natural habitat, at the edge of the water with the head almost out and the back hardly covered. Eleven were found in all in different parts of the terrarium at this time. The others had gone down too far in the loose soil to be located without a more complete overturning of the terrarium than was deemed advisable. Five of the specimens were taken and preserved for later study (stages *B* and *C*). There is no means of knowing the exact date of hatching of each of these specimens, which may have been anywhere from one to five days old; it is very probable, however, that two of them, which were taken from the water (stage *C*) and which were somewhat larger than the others, were hatched earlier, especially as the others (stage *B*) were taken from near the nest.

During the next few days the terrarium was examined from time to time to locate the remaining larvæ and to discover any change in their activities. They never at any time showed the

slightest instinct to go directly to the little pool of water which was now arranged for their reception but would often leave the nest in exactly the opposite direction. In general they made their way, as has been noted above, deeper and deeper into the loose soil until they finally reached the little areas of very shallow water on the surface of the hard soil at the bottom. Under natural conditions the burrows which penetrate the soil in which the *Desmognathus* lives must furnish natural and easy channels for the descent of the larvæ to a level at which the burrows would contain little pools of water. From here it is an easy matter for them to make their way beneath the loose layer of vegetable debris to the open water of the neighboring brook. Possibly an instinct of the larvæ to remain in contact with the body of the mother serves as an additional guide to them, as they would thus naturally follow her movements through the burrows. That the larvæ, like the adults, are thigmotropic, would also lead them to confine their movements to the crevices in the soil; a negative heliotropism may also assist in determining the downward direction of their movements.

On October 5, five more specimens were killed (stage *D*); of these four were found to have been more or less mutilated, having suffered the loss of a tail, or one of the limbs or both tail and limbs. Since it frequently happens that a considerable proportion of larvæ collected from their natural habitat show mutilations similar to these, it is evident that the cause in question is one which is operative under natural conditions. It is possible that the injuries are inflicted by the stronger larvæ upon their weaker brothers, especially as the injured individuals are usually below the average size. It is more probable, however, that the mother, under the pressure of a paucity of food, found her offspring tempting morsels. The fact that two of the brood were shown by the final count to be missing suggests that this conjecture is the correct one, especially as the larvæ themselves are very hardy and will live for weeks in the laboratory under very adverse conditions. I have noted in several instances similar total disappearance of larvæ from terraria in which they had been placed in company with one or two adults, and in two cases have found whole larvæ in the stomachs of adults which were in the terrarium from which larvæ had disappeared.

On October 6 one specimen more was killed (stage *E*), and on October 14 all of the remaining ones which could be found, three in number (stage *F*).

Since by this time the larvæ had not only reached the water in the bottom of the jar but had attained the size and proportions of the aquatic larvæ collected from their natural habitat at this season of the year (cf. Table I., and Graphs I. and II.), it may be

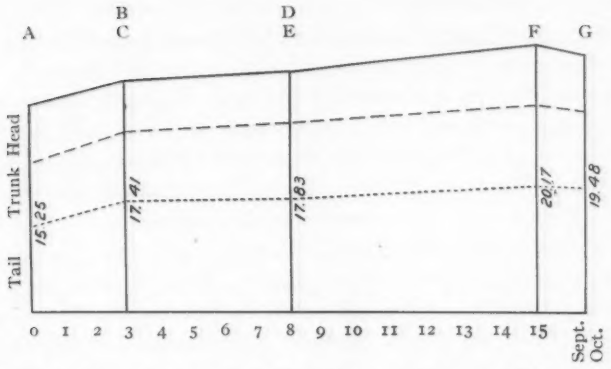
TABLE I.

(The measurements given in this table are approximately those of the head, trunk, and tail, respectively, though, for convenience in measuring, the axilla and the anterior angle which the posterior limbs make with the body were taken as the somewhat arbitrary limits of these regions, cf. Plate II., 11, dotted lines.)

TERRESTRIAL LARVAL PERIOD.

Designation and Age.	Number of Examples.	Column I. Actual Sizes (Cf. Graph I.), Mm.			Column II. Proportionate Lengths of Body Regions (Cf. Graph II.)		
		Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
Stage *A*. 0 days.	2	Head 4.00	4.00	4.00	.258	.267	.262
		Trunk 5.00	5.00	5.00	.323	.333	.328
		Tail 6.50	6.00	6.25	.419	.400	.410
		Total.....15.50	15.00	15.25	1.000	1.000	1.000
Stages *B* and *C*. Average age 3 days.	5	Head 4.00	4.00	4.00	.219	.242	.234
		Trunk 5.50	5.00	5.33	.301	.303	.312
		Tail 8.75	7.50	8.085	.479	.454	.454
		Total.....18.25	16.50	17.415	1.000	1.000	1.000
Stages *D* and *E*. Average age 8 days.	3	Head 4.25	4.00	4.17	.233	.229	.234
		Trunk 5.75	5.50	5.58	.315	.314	.313
		Tail 8.25	8.00	8.08	.452	.457	.453
		Total.....18.25	17.50	17.83	1.000	1.000	1.000
Stage *F*. Average age 15 days.	3	Head 4.50	4.50	4.50	.220–	.225	.223
		Trunk 6.25	6.25	6.17	.305–	.3125	.306
		Tail 9.75	9.25	9.50	.476–	.4625	.471
		Total.....20.50	20.00	20.17	1.000	1.000	1.000

considered that the duration of the terrestrial larval period is approximately 15 or 16 days, the average age of stage *F*. That the duration of the terrestrial period is very variable will, however, be inferred from the fact that in the case of this particular brood a difference in activity led some of the larvæ to the water several



GRAPH I., showing the growth in length during the terrestrial larval period (cf. first column of statistics, Table I.). Lines *A*, *BC*, *DE*, and *F* correspond to the similarly designated stages, the average age of each stage in days being indicated by the number at the bottom of the line and the average length (× 2) by the length of the line. Line *G* shows, for comparison with *F*, the average length of 42 aquatic larvæ collected during the months of September and October.

days in advance of others; and the differences in the distances of nests from the water would introduce still another variable factor.

While the limits of the terrestrial larval stage are somewhat indefinite, the structural changes which take place during this period are very important, and for the study of these changes the above described material may be tabulated as follows (see Plates I. and II.):

Stage A.—Two specimens killed September 25. Just hatched. Average length 15.25 mm.

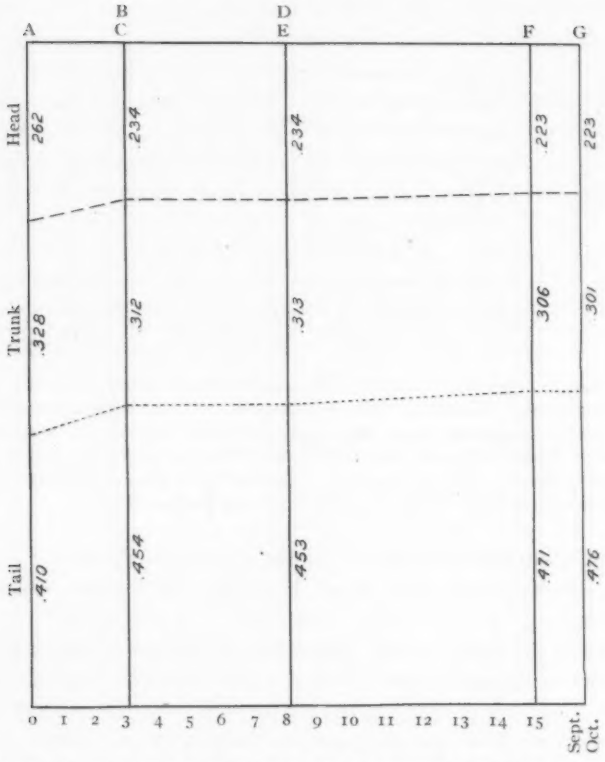
Stage B.—Three specimens killed October 1. Average age 3 days. As these were taken from near the nest the actual age of all of them is probably less. Average length 16.83 mm.

Stage C.—Two specimens killed October 1. Age as in the case of stage *B*, but as these two were taken from the pool of water at a greater distance from the nest, their actual age is probably above the average of 3 days. Average length 18 mm. Average length of *B* and *C* together is 17.415 mm.

Stage D. Five specimens killed October 5. Average age 7½ days. Average length of the two uninjured ones 17.652 mm.

Stage E.—One specimen killed October 6. Average age 8½ days. Length 18.25 mm. Average length of *D* and *E* together is 17.83 mm.

Stage F.—Three specimens killed October 14. Average age 15 days. Average length 20.17 mm.



GRAPH II., showing a comparison of the proportionate lengths of the body regions during the terrestrial larval period (cf. second column of statistics, Table I.). Lines *A*, *BC*, *DE*, and *F* refer to the various terrestrial stages as in Graph I. and line *G* shows, for comparison with *F*, the proportionate lengths of aquatic larvæ collected in September and October.

EXTERNAL FEATURES OF THE TERRESTRIAL LARVA.

The most conspicuous feature of the newly hatched larva (stage *A*, cf. Plate I., 1 and 2)[1] is the bulging form of the abdomen, due to the presence of a considerable mass of as yet unconsumed yolk material. The skin over this region is thin, unpigmented, and transparent, and the yellowish white appearance due to the yolk cells beneath it contrasts strongly with the deeply pigmented surface of the rest of the body. Along the back on each

[1] Plates will be found at the end of the second part of this paper in the next issue of the BIOLOGICAL BULLETIN.

side of the mid-line the general gray pigmentation is interrupted by a row of rather poorly defined, rounded areas of lighter color, in some cases showing a slight brownish tinge. These spots are somewhat irregularly arranged, usually not exactly opposite each other on the two sides. The number on each side in the trunk region varies from 7 to 10, and the series extends nearly throughout the caudal region where it finally leaves off by a gradual diminution in the size of the areas.

The tail is short and shows only a slight beginning of the dorsal and ventral folds which are later to form the tail fin. The gills are well developed and when the specimen is placed in water, they stand out conspicuously from the sides of the body. There are three of these gills upon each side. They are situated upon the lateral surface dorsal to the gill slits, in an oblique line, the most anterior one being more ventral in position and the most posterior one the more dorsal. This latter one is the longest and possesses from five to seven branches some of which bend conspicuously forward; the middle gill has usually five branches, and the ventral one three.

The legs, particularly the posterior ones, are stout and well developed, and are longer in proportion to the length of the trunk region than at any later period of development. Although the quick jerky motions with which the newly hatched larvæ, when disturbed, propel themselves through the loose soil seem to be performed with the whole body rather than with the legs, there are undoubtedly slower movements of the larvæ when left undisturbed, which involve the action of the legs. This greater proportionate size of the hind legs is, it will be remembered, one of the features distinguishing *Desmognathus* from *Spelerpes* larvæ, and is undoubtedly accounted for by the demands of this short terrestrial period preceding the aquatic larval life; since the eggs of *Spelerpes* develop, and the larvæ hatch, in the water, there is no need in this species for the early development of legs as organs of locomotion.

During the period of terrestrial life, important changes take place which result in a decided transformation in the appearance of the larvæ. The yolk protuberance rapidly diminishes, as will be seen by comparing Plate I., 1 to 9, so that by the time

stage *D* is reached (11 days, at the latest, from the time of hatching), practically all external evidence of the presence of yolk has disappeared, though dissection and sectioning both demonstrate the presence of an abundance of yolk granules in the walls of the intestine. With this reduction of the yolk protuberance, the medial edges of the *rectus abdominalis* muscles, widely separated at the time of hatching, gradually approach each other and finally meet in the *linea alba*.

The changes in the shape and appearance of the tail are noteworthy. The tail becomes longer, so that from being at

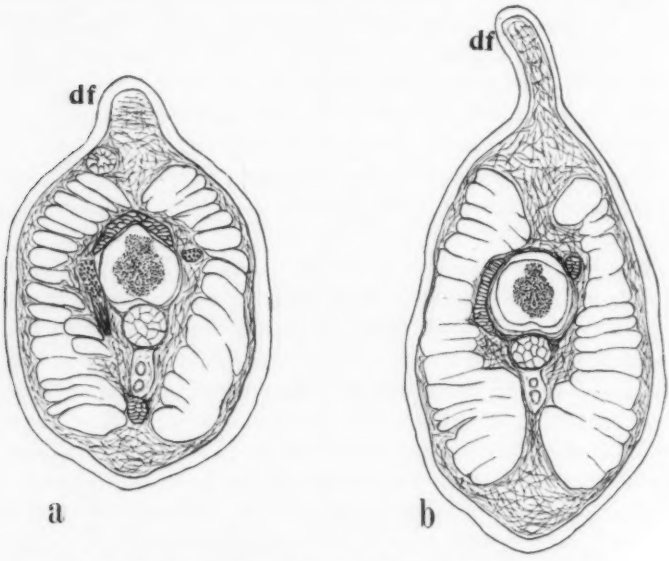


FIG. 7. Cross sections, at approximately the same level, of the tails of (*a*) a newly hatched larva, terrestrial stage *A*, and (*b*) an aquatic larva, showing the difference in proportions, and the development of the dorsal fin (*df*). For the level of the sections cf. Plate I., Figs. 1 and 10. Drawn with Abbé camera. × 60.

the time of hatching only 41 per cent. of the total body length, by the time the larvæ have reached the water and become typical aquatic larvæ (stage *F*, averaging only 15 or 16 days from the time of hatching) this same measurement is found to be over 47 per cent. of the total body length. With the increase in length of

tail comes the change in its shape owing to the development of the median fin both on the dorsal and ventral side. Dorsally it begins at the level of the posterior margin of the pelvic girdle, where a slight depression in the mid-dorsal line may be seen when the animal is viewed from the lateral aspect. This dorsal portion of the fin, which finally in its widest part comes to contribute from one third to two fifths of the entire dorso-ventral dimension of the tail, extends to the extreme tip of the tail, where its continuity with the ventral portion of the fin is interrupted by the slender tip of the vertebral column and its associated muscles. The ventral portion of the fin is neither so wide nor so extensive as the dorsal, and it narrows anteriorly to end at a point somewhat posterior to the cloaca. The increase in length of the tail is the principal factor in the change in length proportions of the body shown in the second column of Table I., and in Graph II. Thus while, during the terrestrial period, the head actually increases only about 12 per cent. in length, and the trunk 23 per cent., the tail increases 52 per cent. (cf. Table I., 1st column). These changes in the tail are obviously a preparation for the aquatic life which is so soon to follow.

Not only do the proportionate lengths of the regions of the body thus alter, but the actual growth is, during the first few days after hatching, very rapid, as will be seen from the 1st column of Table I., and also by comparison of Plate I., 1–9, all drawn to the same scale. Thus at the end of 16 days the total increase in length (20.17 − 15.25 = 4.92 mm.) is 32 per cent. of the length of the body at the time of hatching.

The Alimentary Canal of the Terrestrial Larva.

Internally no structure exhibits more striking and fundamental changes during the terrestrial period than the alimentary canal. In the walls of the alimentary canal at the time of hatching there is still a considerable supply of yolk material, although several days previous to hatching (*i. e.*, in the embryo of 30 days' development, 13 mm. long), the yolk material contained in the cells of the other tissues has been already quite consumed. In the newly hatched larva, indeed, the digestive tract has so far progressed

in its development that œsophagus and stomach have already completed their histogenesis, and the duodenum and rectum practically so; leaving the yolk material in the remaining intestinal region.

The lining of the œsophagus is thrown into longitudinal folds, and is composed of columnar cells of the ciliated type with a large number of active unicellular glands interspersed among them (Fig. 8, *b*). The stomach is sharply differentiated from the œsophagus on the one hand and from the duodenum on the

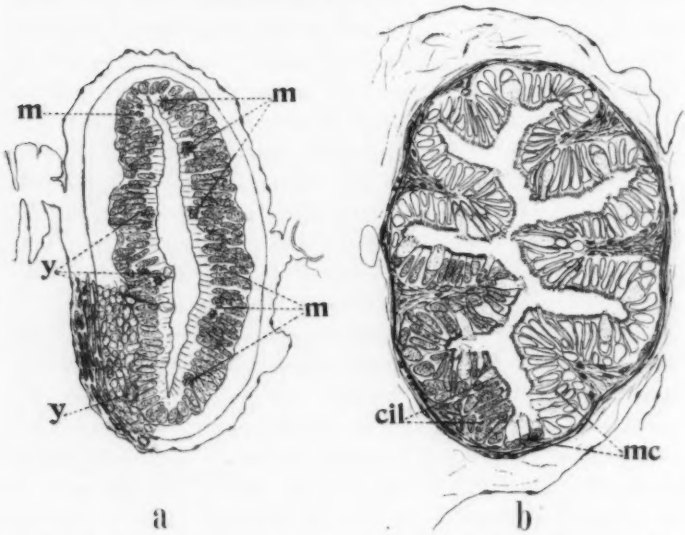


FIG. 8. Cross sections of the œsophagus of (*a*) a 13 mm. embryo, and (*b*) a newly hatched larva, showing the change in the character of the epithelial lining which takes place during the few days previous to hatching. (*a*) *m*, mitosis; *y*, last traces of yolk granules in the epithelial cells; (*b*) *cil*, ciliated epithelial cells; *mc*, mucous cells. Note the absence of folds in (*a*), and of mitosis in (*b*). Drawn with Abbé camera. × 103.

other. It is divided into cardiac and pyloric regions, the former expanded, piriform, and glandular, the latter tubular and far more muscular. In both these regions of the stomach the unicellular type of gland is lacking, but there are numerous multicellular glands of the convoluted tubular type, opening

between the many irregular folds of the lining mucous membrane. The cells of the mucous membrane are sub-columnar in form, with a smooth exposed surface. The final stages of this histogenesis must take place with great rapidity, during the last few days before hatching. In the 13 mm. embryo neither folds, ciliated cells, nor glands of any kind have appeared in the alimentary canal; the stomach is differentiated from the œsoph-

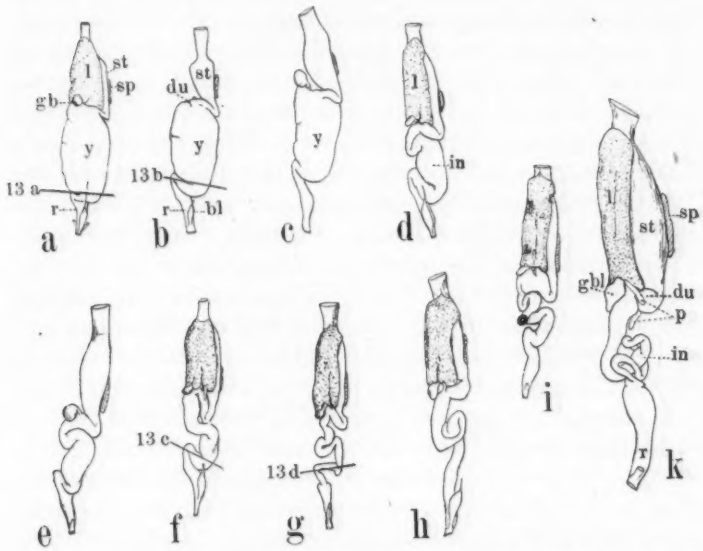


FIG. 9. A series of dissections of the alimentary canals of various stages of *Desmognathus fusca*, showing the gradual consumption of the yolk and the differentiation of the intestine. (*a*) terrestrial larval stage *B*, 3 days old; (*b*) same stage as (*a*) with the liver removed; (*c*) terrestrial larval stage *D*, 7½ days old; (*d*) terrestrial larval stage *F*, 15½ days old; (*e*), (*f*), (*g*), (*h*), aquatic larval stages collected in September and October; (*i*) aquatic larval stage collected in May; (*k*) adult stage, length 45 mm.

Lines 13*a*, 13*b*, 13*c*, and 13*d* show the levels and directions of the respective sections of Fig. 13; *bl*, bladder; *du*, duodenum; *gbl*, gall bladder; *in*, intestine; *l*, liver; *p*, pancreas; *st*, stomach; *sp*, spleen; *r*, rectum; *y*, yolk mass. Drawn with Abbé camera. × 4.

agus, only in being less flattened dorso-ventrally, and containing in its cells a larger amount of yolk; there is an enormous amount of mitosis in progress especially in the œsophageal

region (Fig. 8, *a*), undoubtedly the process of cell proliferation which results in the formation of the longitudinal folds.

With equally rapid strides does the histogenesis of the intestinal region occur during the brief terrestrial period of larval life, until from the large yolk mass which fills and distends the body cavity of the newly hatched larva and furnishes nutriment for the rapidly growing animal, there is developed the entire length of intestine with its convoluted windings, ready for the digestion and absorption of ingested food material.

The changes in the external form of the intestinal region involved in this process may be demonstrated by comparison of the dissections shown in Fig. 9, from which it will be seen that there is gradually moulded out of the intestinal yolk mass a loop forming an *S*-shaped figure which consists of an anterior portion lying upon the right side, into which the duodenum opens, and a posterior portion lying upon the left side, from which the rectum leads. Both of these regions, particularly the left and more posterior loop, are for a considerable time enlarged through the presence of the remaining yolk material as indicated by the condition shown in stage *F*, (Fig. 9, *d*) the oldest of the terrestrial larvæ. Gradually, however, concomitant with the consumption of the last remnants of yolk material and the consequent diminution in the diameter of the intestine, there occurs a still further increase in its length, so that each of the two main loops acquires two or three subsidiary ones variable, of course, in relative position with constantly varying peristaltic conditions (cf. Fig. 9, *e*, *f*, *g*, and *h*).

The last part of this process of differentiation of the intestine is apparently not usually accomplished until after the larva has reached the water, since frequently in the early fall aquatic larvæ are found with the intestine in the condition shown in *d*, *e* and *f*, Fig. 9, exactly similar to the condition found in my oldest terrestrial stage *F*. Food may be ingested many days previous to the final disappearance of the yolk; in fact, the stomach of one specimen of stage *C* (terrestrial larva, average age 3 days and greatest possible age 5 days) contained a small copepod, while there was another in the intestine; and the specimen of stage *D*, from which the dissection shown in Fig. 9, *C*, was made, had 30

copepods in the stomach, and in the stomach of another specimen of the same stage 6 copepods were found.[1] In all the cases which I have examined, of aquatic larvæ in which yolk was still present, feeding has been extensive and fragments of partly digested aquatic animals, in the main copepods and insect larvæ, have been found not only in the stomach but in various regions of the intestinal tract. Whether the early assumption of its digestive function on the part of the alimentary canal has the effect either to retard the final consumption of yolk material through the supply of nutriment from external sources, or to hasten its consumption through the stimulation of the histogenesis of the walls of the canal through use, I am at present unable to say. At any rate the excess of the supply of yolk material over the amount which the larva usually consumes before reaching the water with its ample supply of appropriate food, is undoubtedly an adaptation to a possible prolongation of the terrestrial period such as might easily occur if the eggs happened to be laid at a greater distance than usual from the water, or if an unusually dry season during the period of development of the egg should diminish the water supply in the brook and in the burrows leading to it and thus lengthen the period which might elapse before the larvæ should reach the water.

Internally the differentiation of the intestine from the mass of yolk cells is a somewhat complicated process, the detailed account of which must be reserved for a more technical treatment of the whole subject of the histogenesis of the alimentary canal. Certain salient features of the process may, however, be discussed here.

At the time of hatching the duodenum is in what may be termed the transition stage in its development (*d*, Fig. 12). Anterior to the point where the bile duct leads into the duodenum from the fully formed liver, yolk granules have disappeared, as they have also from the cells of the liver and pancreas and their ducts. Posterior to the opening of the bile duct into the duodenum, there is for some distance a well-defined lumen, but the epithelial cells lining it contain a considerable number of yolk

[1] All of these individuals, though ranking as terrestrial larvæ, happen to be among the more precocious ones which had reached the water, where they had access to food.

granules, although among these cells there have already appeared well-developed mucous cells which are filled with their secretion and are already discharging it into the lumen. The duodenum leads rather abruptly into the right anterior region of the cylindrical yolk mass, beneath the posterior margin of the liver.

In this mass of yolk cells two types of cell may be distinguished, a smaller and a larger (*ys* and *yl*, Fig. 11). The smaller ones lie peripherally, have a spherical or slightly columnar form with a well-defined nucleus usually in the peripheral end of the cell.

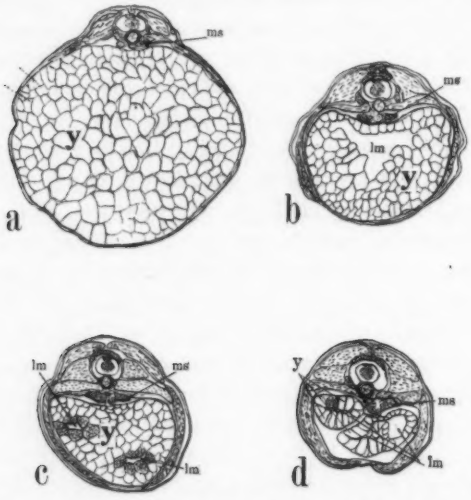


FIG. 10. Cross sections at the same level through the middle of the body of (*a*) a 13 mm. embryo; (*b*) newly hatched larva, terrestrial stage *A* (cf. Fig. 12, *a*, and Plate I., Figs. 1 and 2); (*c*) larva 3 days old, terrestrial stage *C* (cf. Fig. 12, *c*, and Plate I., Figs. 5 and 6); (*d*) larva 15½ days old, terrestrial stage *F* (cf. Fig. 12, *d*, and Plate I., Fig. 9). Note the gradual reduction of the mass of yolk cells (*y*) and the formation of the intestinal lumen (*lm*); *ms*, mesonephros. Enclosure between the dotted lines in (*a*) indicates the location of Fig. 11. Drawn with Abbé camera. × 12½.

The cell walls are definite, the contained yolk granules of varying size, but among them relatively few large ones. The remaining portion of the yolk mass consists of enormous cells which are often multinucleate, with very large, irregular nuclei. The cell walls are not always clearly defined and in many regions seem

to rupture easily. One must, however, remember the great liability to imperfect preservation of the middle region of the yolk, which might account for apparent imperfection of cell walls. Although during the earlier part of development within the egg, these yolk cells of the large type contain both large and small yolk granules with, however, a preponderance of the large ones, by the time the larva hatches practically all of the smaller granules seem to have been consumed, and as a result the large granules alone appear, and these not very closely crowded together. It is from the cells of the smaller type, the

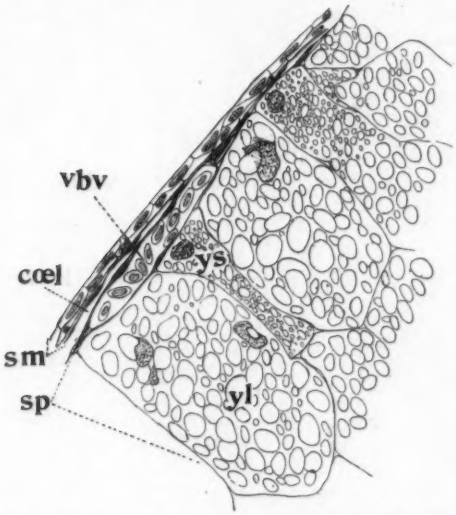


FIG. 11. Detail of region indicated between the dotted lines in Fig. 10 (*a*). *Coel*, coelomic cavity; *sm*, somatopleure; *sp*, splanchnopleure; *vbv*, vitelline blood vessel; *yl*, yolk cells of the larger (nutritive) type; *ys*, yolk cells of the smaller type. Abbé camera. × 273.

peripheral ones, that the future epithelial lining of the intestine will arise, while the large cells which form the bulk of the yolk mass may be regarded as purely nutritive in function, since from these apparently no tissue is derived. If this distinction goes back, as seems probable, to the early differentiation of the cells of the blastula into the peripheral ones containing small yolk granules, and the central mass of cells containing large yolk

granules (Hilton, '09), we see again the approach to an intermediate position of the *Desmognathus* egg between the holoblastic and meroblastic types, this mass of large cells of a purely nutritive character being comparable to the undivided yolk mass of the meroblastic egg. The tardy segmentation of this mass of

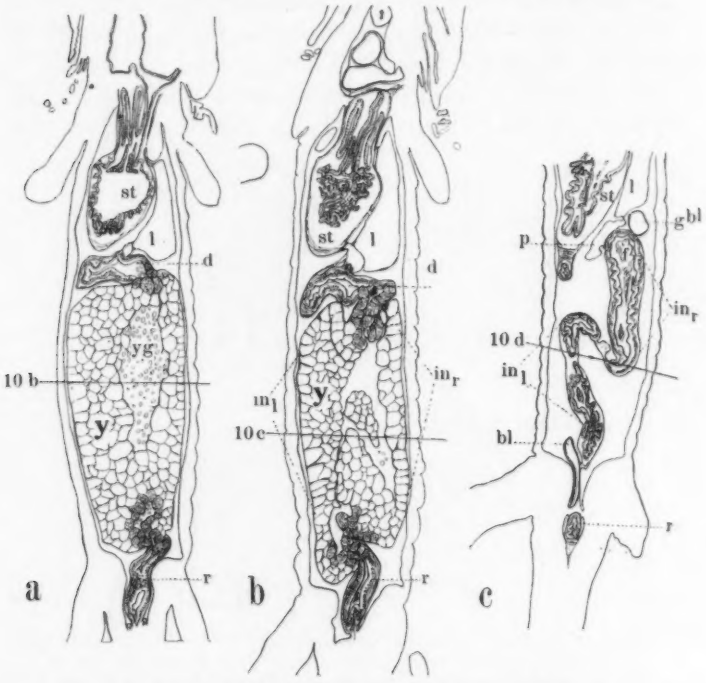


FIG. 12. Horizontal sections (viewed dorsally) of (*a*) terrestrial larva at time of hatching (stage *A*, cf. Plate I., Figs. 1 and 2), (*b*) terrestrial larva 3 days old (stage *C*, cf. Plate I., Figs. 5 and 6), (*c*) terrestrial larva 15½ days old (stage *F*, cf. Plate I., Fig. 9). Lines 10*b*, 10*c*, and 10*d* indicate respectively the levels of the cross sections shown in Fig. 10. *Bl*, bladder; *d*, duodenum; *gbl*, gall bladder; *in*$_l$, left loop of intestine; *in*$_r$, right loop of intestine; *l*, liver; *p*, pancreas; *st*, stomach; *r*, rectum; *y*, yolk mass; *yg*, yolk granules in the lumen of the yolk mass. Drawn with Abbé camera. × 12½.

yolk material, which Hilton pointed out, and the later breaking down of the cell walls of these large nutritive cells, which seems to appear in some cases, corroborate this view.

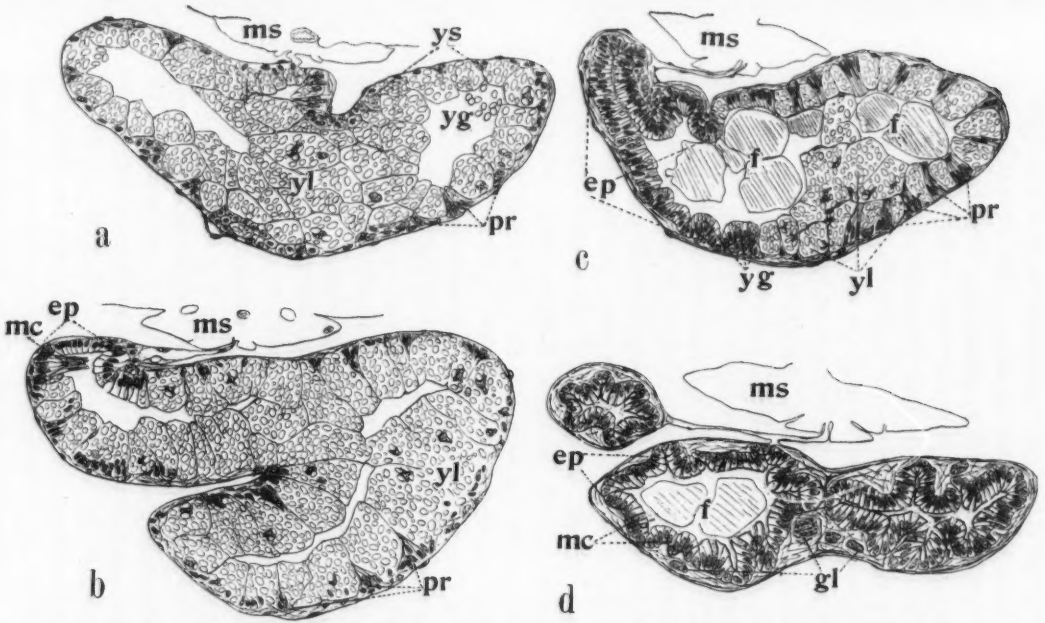


FIG. 13. Sections showing four stages on the differentiation of the posterior part of the intestine from the mass of yolk cells, from cross sections through approximately the same region of the body; (*a*) terrestrial larva at the time of hatching, (*b*) terrestrial larva 3 days old, (*c*) and (*d*) two aquatic larvæ collected in September. The levels and the directions of the sections with respect to the yolk mass and intestine are indicated approximately by lines 13*a*, 13*b*, 13*c*, 13*d*, respectively, in Fig. 9 (*a*), (*b*), (*f*) and (*g*). *Ep*, regions of functional epithelium; *f*, food in the intestinal tract; *gl*, multicellular glands of the intestinal mucous membrane; *mc*, mucous cells; *ms*, mesonephros; *pr*, regions of proliferation of epithelial cells to form future intestinal folds; *yg*, yolk granules; *yl* and *ys*, yolk cells of the large (nutritive) and small (tissue-forming) types respectively. Drawn with Abb écamera. × 60.

Although there is at the time of hatching a considerable cavity within the yolk mass (Figs. 10, *b*, and 12, *a*), its walls are irregular and poorly defined. The anterior region of the cavity is located more upon the right side and communicates with the lumen of the duodenum, while more posteriorly the cavity swings somewhat to the left side, where it finally communicates with a partly differentiated posterior region of the intestine which extends transversely from left to right to lead into the rectum. Thus the *S*-form so soon to appear in the external moulding of the intestine (Fig. 12, *c*) is already indicated in the lumen of the yolk mass.

The further differentiation of the intestine from the mass of yolk cells (Fig. 13) involves a double process consisting of (1) rapid reduction and disappearance of the yolk material contained in the nutritive cells (*yl*), which may from time to time be sloughed off into the lumen, or may be ruptured, thus discharging their contents into the lumen; and (2) the proliferation of the peripheral cells (*pr*), the planes of division being radial with relation to the lumen of the intestine, and resulting in the formation of the anlagen of the future folds of the mucous membrane, in the form of groups of tall columnar cells which, elongating, push their way to the lumen between the remaining nutritive cells (Fig. 13, *c*). These nutritive cells thus come to lie in the furrows between the folds, from which place they gradually disappear. Mucous cells develop early from certain of the peripheral cells, and are of a tall columnar type, corresponding to the form of the other epithelial cells among which they lie. Multicellular glands (*gl*) develop later from a proliferation of cells at the bases of the folds.

Although this differentiation of the intestine progresses almost simultaneously throughout the main part of the yolk mass, it proceeds a little more rapidly at both anterior and posterior ends, than in the middle of the yolk region. Thus at the time of hatching the anterior part of the duodenum and practically all of the rectum are fully differentiated, and the regions of intestine immediately adjoining these are in that stage of development in which the anlagen of the intestinal folds are already formed, a condition which is not reached by the larger portion of the intestine until several days later.

The Skin of the Terrestrial Larva.

The epidermis of the newly hatched larva (Plate IV., 19, 20, 21, and 22) is in the typical larval amphibian condition in that it consists of two layers of cells; these are for the most part subspherical in shape with polygonal outlines when viewed in a section parallel with the external surface. They possess large nuclei which in both layers show a small precentage of mitosis. The cells of the outer layer have a deeply staining cuticular border on the exposed surface, forming all together a continuous cuticular layer (*cu*) covering the external surface. In general over the dorsal and dorso-lateral surfaces the outer layer of the epidermis is the thinner one and its cells are slightly flattened. Over the lateral and ventral surfaces the outer layer is the thicker, the cells here approaching the columnar form, while their nuclei are piriform with the pointed end directed toward the outer surface and often nearly reaching it; in these regions the inner layer is considerably thinner and the nuclei smaller. Over the region which is still distended with yolk, both layers are thin, the cells of the inner one approaching the squamous type. In a few limited regions as, for example, the lateral wall of the body beneath the gills, the whole epidermis is very thin, and the cells of both layers decidedly of the squamous type; as seen in cross section their nuclei alternate, and the whole appearance may thus even simulate a single cell layer.

Beneath the deeper layer of epidermal cells in practically all regions of the body except the snout, there is a dense corium which fits closely to the deeper layer of cells, thus following their contour. This corium is a little thicker than the cuticular layer of the outer cells and is from one eighth to one sixth as thick as the whole epidermis.

In those regions of the body which are pigmented, the dorsal and lateral surfaces, the pigmentation is threefold. The external layer of epidermal cells possesses an intracellular pigmentation in the form of a layer of pigment granules (*pgg*) immediately beneath the cuticular layer and forming a sort of cap over the nucleus. In addition to this intracellular pigmentation there may be seen, between the cells, delicate intercellular ramifying branches (*pgbr*) of pigment cells (*pgc*) of the connective tissue

type, the body of the cell often lying between the two layers of epidermal cells. These branches seem particularly to embrace the cells of the external layer, though occurring between the cells of the deeper layer also. Finally, beneath the corium and closely applied to it, is an interrupted layer of enormous patches of pigment matter which, however, does not seem to penetrate the corium and encroach upon the epidermis. In the unpigmented ventral region all of these forms of pigmentation are lacking.

In comparison with the skin of the 13 mm. embryo the skin of the newly hatched larva of 15.5 mm. impresses one as a fully formed structure (Plate IV., 17 and 18). In many regions of the 13 mm. embryo the epidermis is very thin, and although two layers of cells are present, the nuclei, particularly of the outer layer, are often so widely separated, owing to the flattened form of the cells, as to seem in a vertical section through the skin to be entirely lacking for considerable distances (cf. Fig. 11). There is only the merest trace of cuticular structure in the external border of the outer cells, and practically no corium beneath the lower cells. Branched pigment cells occur both beneath and between the epidermal layers but there is no evidence whatever of intracellular pigmentation of the epidermis. The embryonal character of the epidermis is evidenced by the slightly rounded external contour of the outer layer of cells, and by the large size of the nuclei, which, moreover, present an enormous amount of mitosis (*mt*) in both layers. As might be expected from the fact that the larval two-layered condition has already been reached, the mitotic figures are oriented with their axes parallel with the external surface of the body, and the process is, of course, giving rise to an increase in the number of cells in each layer. This condition of general mitosis contrasts sharply with the limited amount of mitosis in progress in the epidermis of the newly hatched larva, where the figures are, moreover, in late anaphase or telophase.

There occur after hatching but few changes in the skin of the larva to complete this rapid transformation from the embryonal condition to that which is to serve the animal during its eight or more months of larval life (Plate IV., 23, 24, and 25). These

changes, which take place during the brief terrestrial period, involve no mitosis, not even in the rapidly growing tail and tail fin. They consist, rather, in a readjustment and rearrangement of the cells to suit the changes in the shape and proportions of the body, the full quota of cells having been already formed in the mitotic stage preceding hatching. Thus over the growing tail fin the epidermal cells become stretched apart, the cells of the deeper layer alternating with those of the external layer and in some cases actually reaching the external surface. In the ventral region of the body where the area of the surface is rapidly diminishing with the reduction of the yolk, the cells lose their flattened form and become subspherical or even columnar.

Over all parts of the body with limited exceptions, there is a slight increase in the thickness of the skin from various causes. The cuticular layer of the external cells increases slightly in thickness, probably, however, at the expense of the rest of the cell; the dense corium underlying the epidermis becomes a little thicker; the chief increase comes, however, from that change in the inner layer of epidermal cells which is the most conspicuous change in the skin during the terrestrial larval life, the distension of the cells as if from a condition of turgor. This distended condition soon becomes more marked in certain cells than in others, and these eventually form the greatly enlarged, vacuolated cells of the type described by Leydig ('53) and subsequently designated the "Leydig cells" of the epidermis (*lc*). These show a somewhat reduced nucleus and a large cytoplasmic region in which a loose reticulum sometimes appears, but which with most stains gives the effect of a clear area between the nucleus and the thin cell membrane. This enlargement of certain cells naturally increases the thickness of the deeper epidermal layer. The Leydig cells press inward upon the corium and outward upon the cells of the external layer; they seem even to push around and between these latter cells but never to actually reach the external surface. The remaining cells of the deeper layer, which lie between the Leydig cells, become crowded together into a columnar form and reach in most cases from the corium to the outer layer of cells. The differentiation of the Leydig cells has already begun at the time of hatching and is most conspicuous

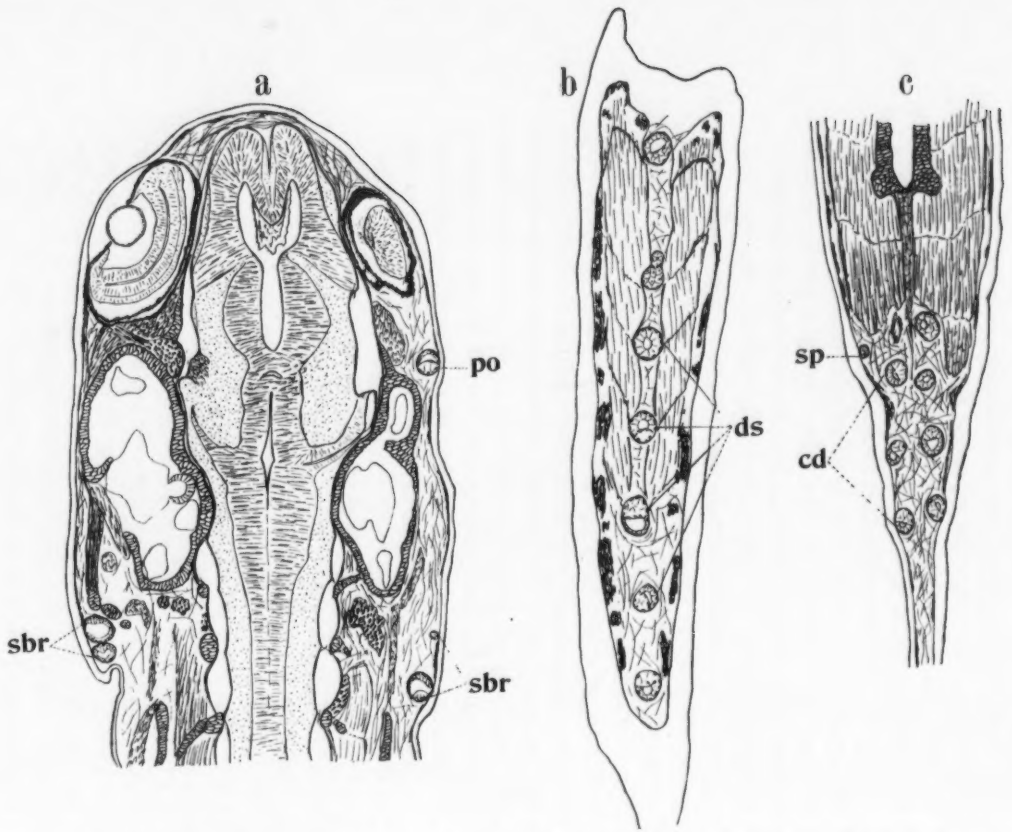


FIG. 14. Horizontal sections of *Desmognathus fusca* larvæ showing the distribution of larval acinous glands; (*a*) through the post-orbital (*po*) and the suprabranchial (*sbr*) groups of glands; (*b*) through seven of the mid-dorsal (*ds*) group; (*c*) through a portion of the double row of the tail (*cd*) at the level of the base of the dorsal fin; also showing a single sporadic gland (*sp*). Drawn with Abbé camera. × 32.5.

along the dorso-lateral regions; it proceeds so rapidly during the few succeeding days that in stage *D* the process is almost completed.

Two sets of highly specialized integumental organs have already made their appearance at the time of hatching. One of these consists of the neuromasts, or sense organs (*sn*) of the lateral line type, which are abundantly distributed over the head, especially the snout, and in two lines which extend along each side of the trunk, while a single line upon each side extends along the tail. These organs, each of which consists of a group of specialized epithelial cells, begin their development long before hatching and at the time of hatching appear to be fully formed so far as the number of cells entering into them is concerned. The surrounding accessory cells probably undergo some further modification.

The other organs to be mentioned are multicellular glands of the alveolar type. They are limited in number and occur in definite locations; a small group of two or three posterior to each eye (the post-orbital group, *po*, Fig. 14, *a*), a group of from one to three over the otic region (the supra-otic group), a larger group of from six to eight on each side of the head posterior to the otic region and dorsal to the gill bushes (the supra-branchial group, *sbr*, Fig. 14, *a*), and a single row approximately segmentally arranged along the middorsal line of the trunk (*ds*, Fig. 14, *b*), becoming, in the transition to the caudal region, a double row in slightly alternating, segmental pairs (*cd*, Fig. 14, *c*) lying along the line of junction of the dorsal finfold and the tail. With the exception of those at the posterior end of the caudal series and a few of the supra-branchial group, these glands are very large, measuring in diameter from two to eight times the thickness of the epidermis; and although they have developed from the epidermis they lie quite beneath both the epidermis and corium within the loose layer of subcutaneous tissue, and open to the exterior by a slender duct which leads through the skin (Fig. 15, *a*). Some of them have already made their appearance in the 18-day embryo as a mass of rapidly dividing epithelial cells, and in the 13 mm. embryo of 30 days' development are

already discharging a granular secretion upon the surface of the skin. Thus the larva hatches fully equipped with a set of actively secreting glands which are to continue their activity throughout the larval life.

(*Concluded in the next issue.*)